FORMULATION AND EVALUATION OF MATRIX TYPE TRANSDERMAL PATCHES OF BENAZEPRIL HYDROCHLORIDE

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MADURAI MEDICAL COLLEGE

MADURAI – 625 020.

MAY - 2012

Dr. Mrs. AJITHADAS ARUNA, M.Pharm., Ph. D.,
Principal,
College of Pharmacy,
Madurai Medical College,
Madurai - 625 020.

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION OF MATRIX TYPE TRANSDERMAL PATCHES OF BENAZEPRIL HYDROCHLORIDE"** submitted by **Miss. R. REVATHI** in partial fulfillment of the requirement for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by her, under the guidance and supervision of **Prof. Mr. A. Abdul Hasan Sathali, M.Pharm., (Ph. D).,** Professor and Head, in the Department of Pharmaceutics, Madurai Medical College, Madurai-20, during the academic year 2011 – 2012. This dissertation is forwarded to the Controller of Examination, The Tamilnadu Dr. M.G.R. Medical University, Chennai.

Place: Madurai **(AJITHADAS ARUNA)**

Date:

Prof. Mr. A. Abdul Hasan Sathali, M.Pharm., (Ph. D).,
Professor & Head,
Department of Pharmaceutics,
College of Pharmacy,
Madurai Medical College,
Madurai-625020.

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I wish her success in all his endeavors.

Place: Madurai **(Prof. Mr. A. Abdul Hasan Sathali)**

Date:

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"I humbly dedicate this little piece of work to almighty"

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CHAPTER I

INTRODUCTION

A drug is any substance or product that is used or is intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient (Tripathi K.D., 2004)

ROUTES OF DRUG ADMINISTRATION (Tripathi K.D., 2004)

Local routes

Topical

Deeper Tissues

Arterial supply

Systemic routes

Oral

Sublingual or buccal

Rectal

Cutaneous – Transdermal therapeutic systems

Inhalation

Nasal

Parenteral

DRUG DELIVERY SYSTEM

For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms, including tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols, and injectables, as drug carriers. This type of drug delivery system is known to provide a prompt release of drug. Therefore, to achieve as well as to maintain the drug concentration within the therapeutically effective range needed for treatment, it is often necessary to take this type of drug delivery system several times a day. This results in a significant fluctuation in drug levels. They have resulted in the development of new techniques for drug delivery.

These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity, and/or targeting the delivery of drug to a tissue.

Sustained release

The term sustained release is known to have existed in the medical and pharmaceutical literature for many decades. It has been constantly used to describe a pharmaceutical dosage form formulated to retard the release of a therapeutic agent such that its appearance in the systemic circulation is delayed and/or prolonged and its plasma profile is sustained in duration. The onset of its pharmacologic action is often delayed, and the duration of its therapeutic effect is sustained.

Controlled release

The term controlled release on the other hand, has a meaning that goes byond the scope of sustained drug action. It also implies a predictability and reproducibility in the drug

release kinetics, which means that the release of drug ingredients from a controlled release drug delivery system proceeds at a rate profile that is not only predictable kinetically, but also reproducible from one unit to another (Chein Y. W., 2005).

ADVANTAGES OF CONTROLLED DRUG DELIVERY SYSTEM

- Decreased incidence and/or intensity of adverse effects and toxicity
- Better drug utilization
- Controlled rate and site of release
- More uniform blood concentration
- Improved patient compliance
- Reduced dosing frequency
- More consistant and prolonged therapeutic effect
- A greater selectivity of pharmacological activity (Jain N. K., 2004)

DISADVANTAGE OF CONTROLLED DRUG DELIVERY SYSTEM

- Increase variability among dosage units
- Stability problems
- Toxicity due to dose dumping
- Increased cost
- More rapid development of tolerance
- Need for additional patient education and counseling (Jain N. K., 2004)

REQUIREMENTS OF CONTROLLED DRUG DELIVERY SYSTEM

- Extended drug action at a predetermined rate
- Localize the drug action
- Target drug action
- Therapeutically based drug release (Remington., 2006)

FACTORS GOVERNING THE DESIGN OF CONTROLLED RELEASE DOSAGE FORMS

Drug related

Aqueous solubility

Partition coefficient

Molecular size

Protein binding

Biological

Absorption

Distribution

Excretion

Duration of action

Margin of safety

Side effects

Physiological

Prolonged drug absorption

Variability in gastro intestinal emptying and motility

Gastro intestinal blood flow

Pharmacokinetic

First pass metabolism

Variability in urinary pH

Enzyme induction/inhibition (Vyas S.P. and Roop K. Khar. 2008)

CHAPTER II

A REVIEW ON TRANSDERMAL DRUG DELIVERY SYSTEM

Transdermal drug delivery systems are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at a controlled rate to the systemic circulation (Monkhouse and Huq, 1988).

FDA approved the first transdermal patch products in 1981. These delivery systems provided the controlled systemic absorption of scopolamine for the prevention of motion sickness (*TransdermScop*, ALZA Corp.) and nitroglycerine for the prevention of angina pectoris associated with coronary artery disease (Transderm-Nitro). Over the last two decades, more than 35 transdermal products have been approved generating sales of $3.2 billion in 2002, which is predicted to rise to $4.5 billion in 2008. More recently, such dosage forms have been developed and/or modified in order to enhance the driving force of drug diffusion (thermodynamic activity) and/or increase the permeability of the skin. These approaches include the use of penetration enhancers, supersaturated systems, prodrugs, liposomes and other vesicles (Bhavna yadav *et al*., 2011).

ADVANTAGES OF TRANSDERMAL DRUG DELIVERY SYSTEM (TDDS)

Transdermal drug delivery systems offer several important advantages over more traditional approaches, including (Sampath kumar K.P. *et al*., 2010)

- Longer duration of action resulting in a reduction in dosing frequency
- Increased convenience to administer drugs which would otherwise require frequent dosing
- Improved bioavailability
- More uniform plasma levels

- Reduced side effects and improved therapy due to maintenance of plasma levels up to the end of the dosing interval
- Flexibility of terminating the drug administration by simply removing the patch from the skin
- Improved patient compliance and comfort via non-invasive, painless and simple application

LIMITATIONS OF TRANSDERMAL DRUG DELIVERY SYSTEM

Some of the greatest disadvantages to transdermal drug delivery are (Sampath kumar K.P. *et al*., 2010)

- Possibility that a local irritation at the site of application
- Erythema, itching, and local edema can be caused by the drug, the adhesive, or other excipients in the patch formulation

STRUCTURE OF THE SKIN

The skin is one of the most extensive and readily accessible organs of the human body. It receives about one-third of the blood circulation through the body (Jain N. K., 2004). The skin is a very effective barrier for the permeation of most xenobiotics. Only a very little drug actually arrives at the site action.

Skin is a multilayered tissue consisting of epidermis, dermis and hypodermis as shown in Figure 1.

Stratum corneum (or) horny layer is the outermost layer of epidermis, which restricts the inward and outward movement of chemical substances. These are compacted, flattened, dehydrated and keratinized cells which are physiologically inactive.

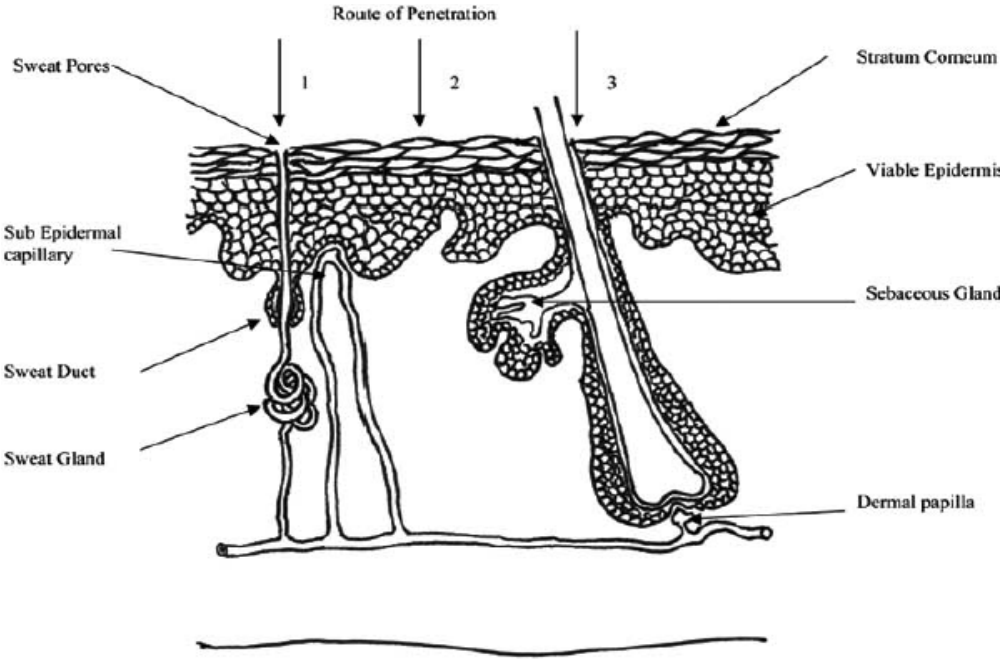


Figure 1 Structure of skin

Stratum corneum has two distinct chemical regions (Jain N. K., 2004) as shown in Figure 2

The mass of intracellular (Transcellular) protein

The intercellular lipoidal medium.

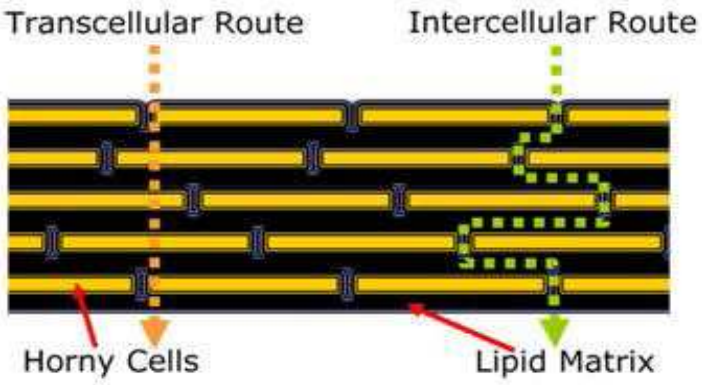


Figure 2 Structure of stratum corneum

The epidermis rests on the much thicker (2000 μm) dermis. The dermis essentially consists of about 80% proteins in a matrix of mucopolysaccharide ground substance

(Jain N. K., 2004). Also contained within the dermis are lymphatics, nerves and epidermal appendages such as hair follicles, sebaceous glands and sweat glands.

PERCUTANEOUS ABSORPTION

Percutaneous absorption involves passive diffusion of substances through the skin. The mechanism of permeation can involve passage through the epidermis itself (transepidermal absorption) or diffusion through shunts, particularly those offered by the relatively widely distributed hair follicles and eccrine glands (transfollicular or shunt pathway absorption) (Jain N. K., 2004).

Transepidermal absorption

Transepidermal (or Transcorneal) penetration includes intracellular and intercellular penetration, hydrophilic drugs generally seen to permeate through intracellular pathway. As stratum corneum hydrates, water accumulates near the outer surface of the protein filaments. Polar molecules appear to pass through this immobilized water. Non polar substances permeate through intercellular penetration. These molecules diffuse into the non-aqueous lipid matrix imbibed between the protein filaments as shown in Figure 3.

Transfollicular (shunt pathway) absorption

In Transappendegeal permeation (shunt pathway) the drug molecule may transverse through the hair follicles, the sebaceous pathway of pilosebaceous apparatus or the aqueous pathway of the salty sweat glands as shown in Figure 3.

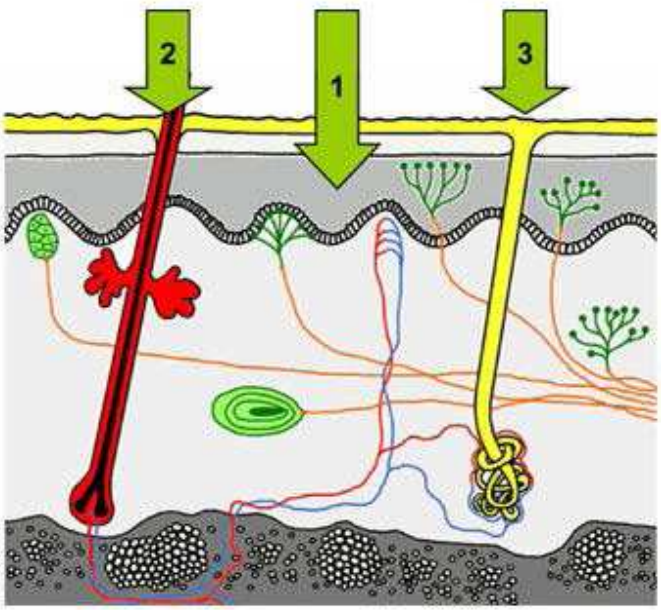


Figure 3 Percutaneous absorption

(1) Across the intact horny layer,

(2) through the hair follicles with the associated sebaceous glands

(3) via the sweat glands

Principles of transdermal permeation

Earlier skin was considered as an impermeable protective barrier, but later investigations were carried out which proved the utility of skin as a route for systemic administration. Skin is the most intensive and readily accessible organ of the body as only a fraction of millimeter of tissue separates its surface from the underlying capillary network. The various steps involved in transport of drug from patch to systemic circulation are as follows (Bhavna yadav *et al*., 2011)

- Diffusion of drug from drug reservoir to stratum corneum
- Sorption by stratum corneum and penetration through viable epidermis
- Uptake of drug by capillary network in the dermal papillary layer

- Effect on target organ.

FACTORS AFFECTING PERMEABILITY

Physiological factors

- ❖ Stratum corneum layer of the skin
- ❖ Anatomic site of application on the body
- ❖ Skin condition and disease
- ❖ Age of the patient
- ❖ Skin metabolism
- ❖ Desquamation (peeling or flaking of the surface of the skin)
- ❖ Skin irritation and sensitization
- ❖ Race

Formulation factors

- ❖ Physical chemistry of transport
- ❖ Vehicles and membrane used
- ❖ Penetration enhancers used
- ❖ Method of application
- ❖ Device used

Physicochemical properties of enhancers

- ❖ Partition coefficient of 1 or greater is required.
- ❖ pH value should be moderate, the flux of ionizable drugs can be affected by changes in pH that alter the ratio of charged and uncharged species and their transdermal permeability.

- Concentration of penetrant higher than solubility, excess solid drug functions as a reservoir and helps in maintaining constant drug concentration for prolonged time (Jalwal P *et al*., 2010).

BASIC COMPONENTS OF TDDS

- Polymer matrix / Drug reservoir
- Drug
- Permeation enhancers
- Pressure sensitive adhesive (PSA)
- Backing laminates
- Release liner
- Other excipients like plasticizers and solvents (Dipen M. Patel *et al*., 2011)

Polymer matrix / Drug reservoir

Polymers are the heart of TDDS, which control the release of the drug from the device. Polymer matrix can be prepared by dispersion of drug in liquid or solid state synthetic polymer base. Polymers used in TDDS should have good stability and compatibility with the drug and other components of the system and they should provide effective release of a drug throughout the device with safe status.

The polymers used for TDDS can be classified as

Natural polymers: e.g. cellulose derivatives, zein, gelatin, shellac, waxes, gums, natural rubber and chitosan *etc*.

Synthetic elastomers: e.g. polybutadiene, hydrin rubber, polyisobutylene, silicon rubber, nitrile, acrylonitrile, neoprene, butyl rubber *etc*.

Synthetic polymers: e.g. polyvinyl alcohol, polyvinylchloride, polyethylene, polypropylene, polyacrylate, polyamide, polyurea, polyvinylpyrrolidone, polymethylmethacrylate *etc*. The polymers like polyethylene glycol, Eudragit, ethyl cellulose, polyvinylpyrrolidone and hydroxypropyl methylcellulose are used as matrix type TDDS. The polymers like ethylvinyl acetate, silicon rubber and polyurethane are used as rate controlling TDDS (Keith AD, 1983).

Drug

The selection of drug for TDDS is based on physicochemical properties of drug. Transdermal drug delivery system is much suitable for drug having (Chung SJ, 1999)

- Extensive first pass metabolism
- Narrow therapeutic window
- Short half-life which causes non-compliance due to frequent dosing
- Dose should be less (mg/day)
- Low molecular weight (less than 500 Daltons)
- Adequate solubility in oil and water
- Low melting point (less than 200°C)

Permeation enhancers

These compounds are useful to increase permeability of stratum corneum by interacting with structural components of stratum corneum *i.e.,* proteins or lipids to attain higher therapeutic levels of the drug (Williams AC., Barry BW, 2004). They alter the protein and lipid packaging of stratum corneum, thus chemically modifying the barrier functions leading to increased permeability (Karande P *et al*., 2005).

Some examples are

Dimethyl sulfoxide, Propylene glycol, 2-Pyrrolidone, Isopropyl myristate, Laurocapram (Azone), Sodium lauryl sulfate, Sorbitan monolaurate, Pluronic, Cardamom oil, Caraway oil, Lemon oil, Menthol, d limonene, Linoleic acid.

Pressure sensitive adhesives (PSA)

The pressure-sensitive adhesive (PSA) affixes the Transdermal drug delivery system firmly to the skin. It should adhere with not more than applied finger pressure, be aggressively and permanently tacky and exert a strong holding force. Additionally, it should be removable from the smooth surface without leaving a residue (Pocius AV *et al*., 1991) Adhesives must be skin-compatible, causing minimal irritation or sensitization, and removable without inflicting physical trauma or leaving residue. In addition, they must be able to dissolve drug and excipient in quantities sufficient for the desired pharmacological effect without losing their adhesive properties and skin tolerability.

PSAs used in commercially available transdermal systems includes

Polyacrylate

Polyisobutylene

polysiloxane 30

Polyacrylates

In general, all acrylic adhesives are polar in character, allowing them to absorb moisture readily and to maintain adhesion to wet skin. They also dissolve most drugs well, enabling high drug loading of polyacrylate matrices.

Polyisobutylenes (PIBs)

Polyisobutylenes (PIBs), in contrast, are characterized by a low solvent capacity for drugs. PIBs are often used in membrane-controlled systems where the initial burst of drug released from the adhesive layer should be limited. PIB-based adhesives are mixtures of high and low molecular weight polymers, which provide cohesion and tackiness, respectively. By adjusting the composition of the PIB formulation, cold flow and adhesiveness can be customized for each system.

Silicone

Silicone, adhesives are characterized by low allergenicity. Similar to PIBs, silicones dissolve most drugs poorly and regulate tackiness and cohesion through polymer size. Molecular weight of silicones, however, can be hard to control during storage of drug-adhesive formulations, since drugs containing amine groups can catalyze further polymerization in silicone adhesives retaining residual silanol groups. To address this problem, special silicones have been developed that are rendered resistant to amine-catalyzed condensation through end-capping of silanol functional groups.

Hot Melt Pressure Sensitive Adhesives (HMPSA)

Melt to a viscosity suitable for coating, but when they are cooled they generally stay in a flow less state. They are thermoplastic in nature.

- Compounded HMPSA
- Ethylene vinyl acetate copolymers
- Paraffin waxes
- Low density polypropylene
- Styrene-butadiene copolymers

- Ethylene-ethacrylate copolymers
- Uncompounded HMPSA
- Polyesters
- Polyamides
- Polyurethanes

Backing laminate

Backing materials must be flexible while possessing good tensile strength. Commonly used materials are;

- polyolefin's
- polyesters
- Elastomers in clear, pigmented, or metalized form

Elastomeric materials such as low-density polyethylene conform more readily to skin movement and provide better adhesion than less compliant materials such as polyester. Backing materials should also have low water vapor transmission rates to promote increased skin hydration and, thus, greater skin permeability. In systems containing drug within a liquid or gel, the backing material must be heat-sealable to allow fluid-tight packaging of the drug reservoir using a process known as form-fill-seal. The most comfortable backing will be the one that exhibits lowest modulus or high flexibility, good oxygen transmission and a high moisture vapor transmission rate (Pfister WR., Hsieh DS., 1990).

Examples of some backing materials

- vinyl
- polyester films
- Polyester-polypropylene films

- Polypropylene resin
- Polyethylene resin
- Polyurethylene
- Co Tran 9722 film
- Ethylene-vinyl acetate
- Aluminized plastic laminate

Release Liner

During storage the patch is covered by a protective liner that is removed and discharged immediately before the application of the patch to skin. It is therefore regarded as a part of the primary packaging material rather than a part of dosage form for delivering the drug. However, as the liner is in intimate contact with the delivery system, it should comply with specific requirements regarding chemical inertness and permeation to the drug, penetration enhancer and water. Typically, release liner is composed of a base layer which may be non-occlusive (*e.g*. paper fabric) or occlusive (*e.g*. polyethylene, polyvinylchloride) and a release coating layer made up of silicon or teflon. Other materials used for TDDS release liner include polyester foil and metalized laminates.

Other excipients

Solvents

Solvents such as chloroform, methanol, acetone, isopropanol and dichloromethane are used to prepare drug reservoir.

Plasticizers

Plasticizers such as dibutylphthalate, triethylcitrate, polyethylene glycol and propylene glycol are added to provide plasticity to the transdermal patch.

METHODS OF PREPARATION OF TDDS

Asymmetric TPX membrane method

A prototype patch can be fabricated for this a heat sealable polyester film (type 1009, 3m) with a concave of 1cm diameter will be used as the backing membrane. Drug sample is dispensed into the concave membrane, covered by a TPX {poly (4-methyl-1 pentene)} asymmetric membrane, and sealed by an adhesive. [(Asymmetric TPX membrane preparation): These are fabricated by using the dry/wet inversion process. TPX is dissolved in a mixture of solvent (cyclohexane) and nonsolvent additives at 60°c to form a polymer solution. The polymer solution is kept at 40°C for 24 hrs and cast on a glass plate to a pre-determined thickness with a gardner knife. After that the casting film is evaporated at 50°C for 30 sec, then the glass plate is to be immersed immediately in coagulation bath [maintained the temperature at 25°C]. After 10 minutes of immersion, the membrane can be removed, air dry in a circulation oven at 50°C for 12 hrs].

Circular teflon mould method

Solutions containing polymers in various ratios are used in an organic solvent. Calculated amount of drug is dissolved in half the quantity of same organic solvent. Enhancers in different concentrations are dissolved in the other half of the organic solvent and then added. Di-N-butyl phthalate is added as a plasticizer into drug polymer solution. The total contents are to be stirred for 12 hrs and then poured into a circular teflon mould. The moulds are to be placed on a leveled surface and covered with inverted funnel to control solvent vaporization in a laminar flow hood model with an air speed of 0.5 m/s. The solvent is allowed to evaporate for 24 hrs. The dried films are to be stored for another 24 hrs at

25±0.5°C in a desiccators containing silica gel before evaluation to eliminate aging effects. The type films are to be evaluated within one week of their preparation.

Mercury substrate method

In this method drug is dissolved in polymer solution along with plasticizer. The above solution is to be stirred for 10 - 15 minutes to produce a homogenous dispersion and poured in to a leveled mercury surface, covered with inverted funnel to control solvent evaporation.

By using "IPM membranes" method

In this method drug is dispersed in a mixture of water and propylene glycol containing carbomer 940 polymers and stirred for 12 hrs in magnetic stirrer. The dispersion is to be neutralized and made viscous by the addition of triethanolamine. Buffer pH 7.4 can be used in order to obtain solution gel, if the drug solubility in aqueous solution is very poor. The formed gel will be incorporated in the IPM membrane.

By using "EVAC membranes" method

In order to prepare the target transdermal therapeutic system, 1% carbopol reservoir gel, polyethylene (PE), ethylene vinyl acetate copolymer (EVAC) membranes can be used as rate control membranes. If the drug is not soluble in water, propylene glycol is used for the preparation of gel. Drug is dissolved in propylene glycol; carbopol resin will be added to the above solution and neutralized by using 5% w/w sodium hydroxide solution. The drug (in gel form) is placed on a sheet of backing layer covering the specified area. A rate controlling membrane will be placed over the gel and the edges will be sealed by heat to obtain a leak proof device.

Aluminium backed adhesive film method

Transdermal drug delivery system may produce unstable matrices if the loading dose is greater than 10 mg. Aluminium backed adhesive film method is a suitable one. For preparation of same, chloroform is choice of solvent, because most of the drugs as well as adhesive are soluble in chloroform. The drug is dissolved in chloroform and adhesive material will be added to the drug solution and dissolved. A custom made aluminium former is lined with aluminium foil and the ends blanked off with tightly fitting cork blocks.

Preparation of TDDS by using Proliposomes

The proliposomes are prepared by carrier method using film deposition technique. From the earlier reference drug and lecithin in the ratio of 0.1:2.0 can be used as an optimized one. The proliposomes are prepared by taking 5mg of mannitol powder in a 100 ml round bottom flask which is kept at 60-70°c temperature and the flask is rotated at 80-90 rpm and dried the mannitol at vacuum for 30 minutes. After drying, the temperature of the water bath is adjusted to 20-30°C. Drug and lecithin are dissolved in a suitable organic solvent mixture, a 0.5ml aliquot of the organic solution is introduced into the round bottomed flask at 37°C, after complete drying second aliquots (0.5ml) of the solution is to be added. After the last loading, the flask containing proliposomes are connected in a lyophilizer and subsequently drug loaded mannitol powders (proliposomes) are placed in a desiccator over night and then sieved through 100 mesh. The collected powder is transferred into a glass bottle and stored at the freeze temperature until characterization.

By using free film method

Free film of cellulose acetate is prepared by casting on mercury surface. A polymer solution 2% w/w is to be prepared by using chloroform. Plasticizers are to be incorporated at

a concentration of 40% w/w of polymer weight. Five ml of polymer solution was poured in a glass ring which is placed over the mercury surface in a glass petridish. The rate of evaporation of the solvent is controlled by placing an inverted funnel over the petridish. The film formation is noted by observing the mercury surface after complete evaporation of the solvent. The dry film will be separated out and stored between the sheets of wax paper in a desiccator until use. Free films of different thickness can be prepared by changing the volume of the polymer solution (J. Ashok kumar *et al*., 2009).

TYPES OF TRANSDERMAL PATCHES

Polymer membrane permeation-controlled TDDS

In this system, the drug reservoir is embedded between an impervious backing layer and a rate controlling membrane (Figure 4). The drug releases only through the rate controlling membrane, which can be micro porous or non-porous. In the drug reservoir compartment, the drug can be in the form of a solution, suspension, or gel or dispersed in solid polymer matrix. On the outer surface of the polymeric membrane a thin layer of drug-compatible, hypoallergenic adhesive polymer can be applied. The rate of drug release from this type of Transdermal drug delivery system can be tailored by varying the polymer composition, permeability coefficient and thickness of the rate controlling membrane.

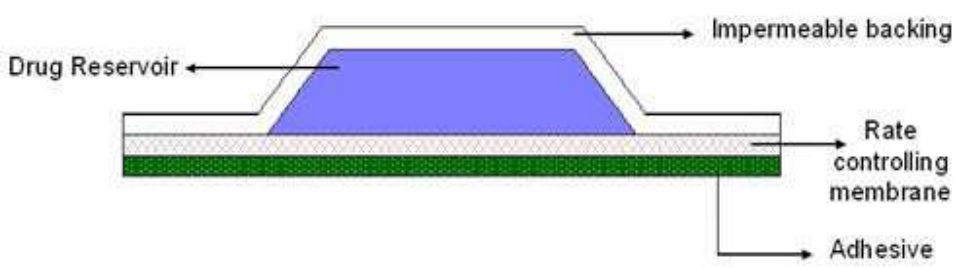


Figure 4: Polymer membrane permeation-controlled TDDS

Examples

TransdermScop (Scopolamine) for 3 days protection of motion sickness and TransdermNitro (Nitroglycerine) for once a day medication of angina pectoris.

Adhesive diffusion controlled TDDS

The drug reservoir is formed by dispersing the drug in an adhesive polymer and then spreading the medicated polymer adhesive by solvent casting or by melting the adhesive (in case of hot-melt adhesives) onto an impervious backing layer (Figure 5). The drug reservoir layer is then covered by a non-medicated rate controlling adhesive polymer of constant thickness to produce an adhesive diffusion controlling drug delivery system.

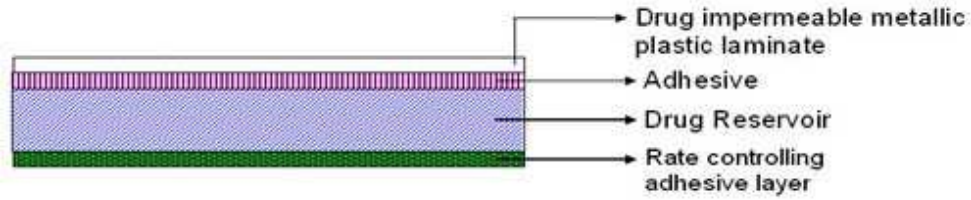


Figure 5: Adhesive diffusion controlled TDDS

Example

Deponit (Nitroglycerine) for once a day medication of angina pectoris.

Matrix diffusion controlled TDDS

The drug is dispersed homogeneously in a hydrophilic or lipophilic polymer matrix. This drug containing polymer disk then is fixed onto an occlusive base plate in a compartment fabricated from a drug-impermeable backing layer (Figure 6). Instead of applying the adhesive on the face of the drug reservoir, it is spread along the circumference to form a strip of adhesive rim.

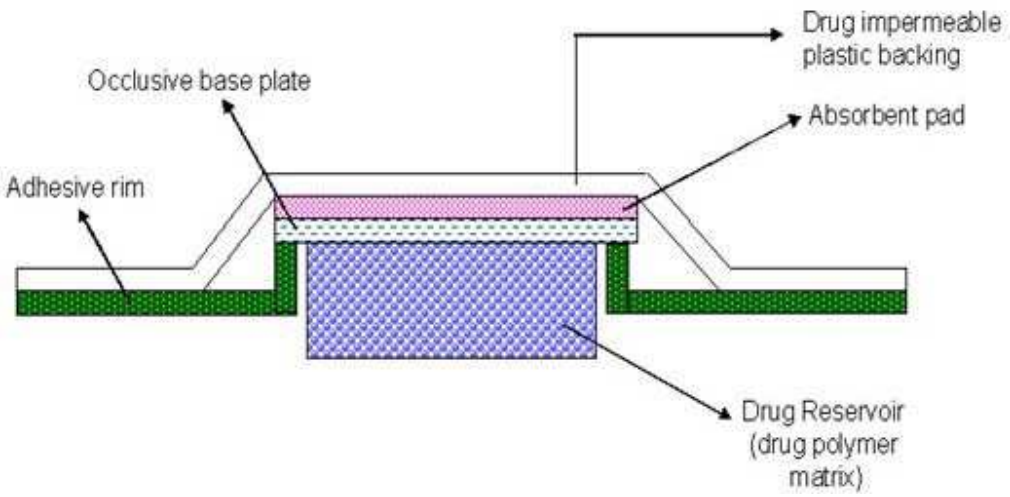


Figure 6: Matrix diffusion controlled TDDS

Example

Nitro Dur (Nitroglycerine) used for once a day medication of angina pectoris.

Microreservoir controlled TDDS

This drug delivery system is a combination of reservoir and matrix-dispersion systems (Figure 7). The drug reservoir is formed by first suspending the drug in an aqueous solution of water-soluble polymer and then dispersing the solution homogeneously in a lipophilic polymer to form thousands of unreachable, microscopic spheres of drug reservoirs. The thermodynamically unstable dispersion is stabilized quickly by immediately cross linking the polymer in situ. A transdermal system therapeutic system thus formed as a medicated disc positioned at the center and surrounded by an adhesive rim **(**Dipen M. Patel and Kavitha K., 2010).

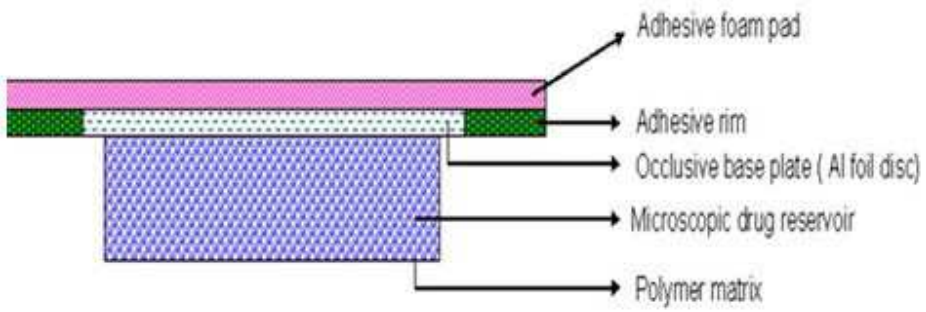


Figure 7 : Microreservoir controlled TDDS

Example

Nitro-dur® System (Nitroglycerin) for once a day treatment of angina pectoris.

EVALUATION OF TRANSDERMAL PATCHES

Transdermal patches have been developed to improve clinical efficacy of the drug and to enhance patient compliance by delivering smaller amount of drug at a predetermined rate. This makes evaluation studies even more important in order to ensure their desired performance and reproducibility under the specified environmental conditions (Bhavna Yadav *et al*., 2011). These studies are predictive of transdermal dosage forms and can be classified into following types:

- Evaluation of adhesive
- Physicochemical evaluation
- In vitro drug release evaluation
- Effect of skin uptake and metabolism
- In vivo evaluation
- Cutaneous toxicological evaluations

Evaluation of adhesive

Pressure sensitive adhesives are evaluated for the following properties (Jain N. K., 2004)

1) Peel adhesion properties

2) Tack properties

- Thumb tack test
- Rolling ball tack test
- Quick stick test (peel tack test)
- Probe tack test

3) Shear strength properties

Physicochemical evaluation (Bhavna yadav *et al*., 2011)

- Physical Appearance
- Weight variation
- Thickness of the patch
- Folding Endurance
- Flatness
- Percentage Moisture Content
- Estimation of drug content

In vitro drug release evaluation (Bhavna yadav *et al*., 2011)

Drug release mechanisms and kinetics are two characteristics of the dosage forms which play an important role in describing the drug dissolution profile from a controlled release dosage forms and hence there in vivo performance. The dissolution data is fitted to

these models and the best fit is obtained to describe the release mechanism of the drug. There are various methods available for determination of drug release rate of TDDS.

- The Paddle over Disc (USP apparatus 5/ PhEur 2.9.4.1)

This method is identical to the USP paddle dissolution apparatus, except that the transdermal system is attached to a disc or cell resting at the bottom of the vessel which contains medium at 32 ±5°C.

- The Cylinder modified USP Basket (USP apparatus 6 / PhEur 2.9.4.3)

This method is similar to the USP basket type dissolution apparatus, except that the system is attached to the surface of a hollow cylinder immersed in medium at 32 ± 5°C.

- The reciprocating disc (USP apparatus 7)

In this method patches attached to holders are oscillated in small volumes of medium, allowing the apparatus to be useful for systems delivering low concentration of drug. In addition paddle over extraction cell method (PhEur 2.9.4.2) may be used.

Effect of skin uptake and metabolism

For studying in vitro skin uptake and metabolism of drug, a piece of full thickness skin (human cadaver skin) or stripped skin freshly excised from a hairless mouse, 5 – 7 week old, was mounted between the two compartments of each V – C permeation cell. It was mounted in such a way that either the stratum corneum or the dermis faced the drug solution and the other side of the skin was protected with impermeable aluminium foil. The compartment with the skin surface covered with aluminium foil remained empty. Both compartments were maintained isothermally at 37^0 C. Samples were withdrawn from solution compartment at predetermined times and assayed for drug and any possible metabolites (Jain N. K., 2004).

In vivo evaluation (Bhavna yadav *et al*., 2011)

- Animal models

  The most common animal species used for evaluating transdermal drug delivery system are mouse, hairless rat, hairless dog, hairless rhesus monkey, rabbit, guinea pig etc.

- Human models

The final stage of the development of a transdermal device involves collection of pharmacokinetic and pharmacodynamic data following application of the patch to human volunteers. Clinical trials have been conducted to assess the efficacy, risk involved, side effects, patient compliance etc.

Cutaneous toxicological evaluations

a) Contact dermatitis

- Contact irritant dermatits

  Ten – day primary irritation test

  Twenty – one day irritation test

  Laser Doppler

  Evaporative water loss measurements

- Contact allergic dermatitis

b) Growth of microorganisms

- Localized superficial infections
- Miliaria

CHAPTER III

LITERATURE REVIEW

Rupesh V. Chikhale *et al*., 2011, analyzed design, formulation and evaluation of transdermal drug delivery system of budesonide. In this study polymeric films containing ER L100: ER S 100: drug (7:3:1, 7:2:1) and EC: PVP: drug (7:3:1, 7:2:1) were selected for transdermal administration based on evaluation studies. The polymeric films were prepared by mercury substrate method employing PEG-400 as plasticizer. Urea and Dimethyl sulphoxide were used as penetration enhancer. In vitro drug permeation, moisture absorption and water vapor transmission studies were carried out on these patches.

Jia-You Fang *et al*., 2011, developed enhancement techniques for improving 5-amino levulinic acid delivery through the skin. In this study enhancement of 5-aminolevulinic acid skin penetration can be achieved by physical methods, such as iontophoresis, laser, micro needles, ultrasound, and by adding chemical penetration enhancers such as DMSO, oleic acid, and others whereas some researchers used lipophilic alpha liphoic acid derivatives and different vehicles to improve the transdermal delivery of 5-aminolevulinic acid.

Jatin Kumar Pruthi *et al.*, 2011, developed and evaluated matrix type transdermal patch of ethinylestradiol and medroxy progesterone acetate for anti-implantation activity in female wistar rats. From this result they suggested that transdermal formulation aimed for postcoital antifertility activity has been successfully developed in female wistar rats.

Hoo-Kyun Choi *et al*., 2011, studied influence of formulation variables in transdermal drug delivery system containing zolmitriptan. In this study, effects of different formulation variables including pressure sensitive adhesive, thickness of the matrix, solvent system, inclusion of crystallization inhibitor, loading amount of drug and enhancers on the transdermal absorption of zolmitriptan were investigated. This study suggests that matrix based transdermal dosage form of zolmitriptan could be explored for the management of migraine.

Venkateswara Raoj *et al.,* 2010, developed matrix type transdermal patches of lercanidipine hydrochloride & analyzed physicochemical and In-vitro characterization. The purpose of the study was to select a suitable formulation for the development of transdermal drug delivery system of lercanidipine and to determine the effect of penetration enhancer, limonene on drug permeation. The transdermal patches were prepared by solvent evaporation technique. In conclusion the patches composed of Eudragit RL, HPMC (1.5:8.5) with 8% V/W limonene at penetration enhancer may be selected for the development of transdermal drug delivery system of lercanidipine for potential therapeutic use by using a suitable adhesive layer and backing membrane.

Shashikant D. Barhate *et al.,* 2010, evaluated in vitro permeation studies of indapamide from transdermal films. Transdermal films were prepared by dissolving Eudragit RS100, lecuric acid, adipic acid, polyvinyl alcohol, sorbitol & indapamide in water. In vitro permeation experiment was performed in Franz diffusion cell. Result of this study show that ER S100 & polyvinyl acetate in 1:2 proportions proved to be better composition for preparation of transdermal film.

Saphie Martel *et al.*, 2010, analyzed physicochemical profile & invitro permeation behavior of a new class of non-steroidal anti-inflammatory drug candidates. In this work lipophilicity & permeability profile of SA derivates were performed to evaluate their ADME properties related to oral or transdermal delivery. All the tested compounds showed potential good passive permeation through GIT & all through percutaneous barrier which could be a way to avoid the first hepatic pass.

Nerner Weitschier *et al*., 2010, analysed adhesion testing of transdermal matrix patches with a probe tack test invitro and in vivo evaluation. In this study twelve different types of polyacrylate pressure sensitive adhesive have been characterized using the probe tack test. In addition to in vitro characterization the in vivo adhesive properties were investigated in a double blinded and randomized wear study by 8 volunteers for a period of 7 days of wear. The invitro data correlate mostly with the in vivo performance of the tested adhesive after 7 days. Accordingly probe tack test could be a helpful tool during the development of transdermal patches.

Naohire Nishida *et al*., 2010, developed and evaluated monolithic drug in adhesive patch for valsartan. In this study to improve the penetration of valsartan in the patch, several chemical penetration enhancers were investigated by in vitro hairless mouse and Yucatan micro pig skin permeation studies. The plasma concentration time profile of valsartan after the patch was applied in human was estimated by a convolution technique. The results of the invitro Yucatan micro pig study, which indicated that the concentration of valsartan could be sufficient to produces a pharmacological effects.

Meenakshi Bharkatiya *et al.,* 2010, Analysed & developed transdermal patches of metroprolol tartarate. In this matrix type patches were prepared by solvent casting method, employing a mercury substrate by using combination of ethylcellulose polyvinyl pyrolidone (PVP) & Eudragit RL100-PVP in different proportions. The result showed that in vitro drug permeation followed Higuchi kinetics, the diffusion coefficient value indicated Fickian transport diffusion.

Bharkatiya M *et al*., 2010, Designed & characterized drug free patches for transdermal application. The aim of this study was to develop drug free polymeric patches using different polymers, to study the effect of different plasticizers on physicochemical properties of the patches, to explore their feasibility for transdermal application. Result of this study showed that the strength & folding endurance of the patches prepared with dibutylphthalate & plasticizer was high compared with propylene glycol & polyethylene glycol. They concluded that plasticizers have a significant influence on the mechanical properties of the transdermal patches.

Liang Fang *et al*., 2010, developed transdermal patches for site-specific delivery of anastazole, invitro & local tissue disposition evaluation. In this study different adhesive matrixes, permeation enhancers & amounts of anastrazole were investigated for promoting the passage of anastrazole through the skin of rats in vitro. These findings show that anastrazole transdermal patches are an appropriate delivery system for application to the breast tumor region for site, specific drug delivery to obtain a high local drug concentration.

Gasem K. A. M *et al.,* 2010, evaluated the effect of different enhancers on the transdermal permeation of insulin analog. The results of this study lead to the conclusion that no specific chemical permeation enhancer (CPE) functional group are directly responsible for enhanced insulin permeation rather permeation enhancements is produced by molecules that exhibit

positive log k_0/w value and possess at least one hydrogen donor or acceptor, with the exception of toluene among the 22 CPES considered.

Alfons Schnitzler *et al*., 2010, evaluated high compliance with rotigotine transdermal patch in the treatment of idiopathic Parkinson's disease. The purpose of this study was the non ergot dopamine against rotigotine has been formulated in a once daily transdermal patch for 24 hours application which ensures continuous rotigotine release over 24 hours. This open prospective, non interventional study investigated compliance with the patch under clinical practice conditions. In conclusion rotigotine transdermal patch was associated with high compliance in patients with Parkinson's disease under clinical practice condition.

Wael Samy *et al*., 2009, evaluated the mechanical properties and drug release of cross linked Eudragit films containing Metronidazole. From this study they conclude that increasing the concentration of either cohesion promoter or the plasticizer gave more significant effect on the mechanical properties of tested films.

Jamakandi V G *et al*., 2009, formulated, characterized and evaluated matrix-type transdermal patches of a model antihypertensive drug. This investigation was aimed to evaluating the possibility of using different polymeric grades of hydroxy propyl methyl cellulose (6 cps, 15 cps, and k_4m) for the development of transdermal drug delivery systems of nicorandil, an antianginal drug. They concluded that transdermal patch with HPMC 6 cps and 6%W/V DMSO as permeation enhancer showed maximum of the drug release and offered least resistance to the movement of the drug molecule due to its high hydrophilic nature and high water permeability value to water.

Shashikant D. Barhaten *et al.,* 2009, developed transdermal drug delivery system of ketoprofen. In this study ketoprofen transdermal patches was prepared by mercury substrate method using polymer Eudragit RS100, Eudragit RL100, HPMC K100M, HPMC E_5 and HPMC K_{4M}, Propylene glycol and oleic acid used as a skin permeation enhancer and dibutylphalate, PEG 400 used as a plasticizer. From the results, the order of permeation of ketoprofen from different polymeric membranes was found to be ERL100: PVP > ERS100: PVP > EC > CA > EC: PVP > CA: PVP. The values of coefficient of correlation for zero order model suggested controlled release of ketoprofen from fabricated transdermal patches.

Liang Fang *et al*., 2009, developed a drug in adhesive transdermal patch for s-amlodipine free base. The objective of this study was to develop and to evaluate a drug in adhesive transdermal patch for s-amlodipine. The effects of the type of adhesive and the content of permeation enhancers on s-amlodipine free base transport across excised rat skin were evaluated. In conclusion, the present data confirm the feasibility of developing s-amlodipine transdermal patch to provide plasma levels for 3 days in rats.

Katerianal Brychtova *et al*., 2009, analyzed physico-chemical properties and penetration activity of alkyl -6 - (2, 5-dioxopyrolidin-1-yl) - 2- (2-oxopyrrolidin-1-yl) hexanoates as potential transdermal penetration enhancers. In this study all the prepared compounds were analyzed using reverse phase high performance liquid chromatography method for the lipophilicity measurement and their lipophilicity was determined. The relationship between the lipophilicity and the chemical structure of the studied compounds as were as the relationships between their chemical structure and transdermal penetration activity are mentioned.

Jianping Liu *et al.,* 2009, developed double layer weekly sustained release transdermal patch containing gestodene and ethinyl estradiol. In this study different polymeric combinations were used to develop polymeric films matrix type transdermal patch. Double layer TDDS could sustain the steady permeation fluxes of drugs for 7 days. As sole enhancer PG could increase the permeation fluxes of drugs. The result obtained suggested that double layer weekly sustained release matrix transdermal patch could be a promising delivery system for non-oral contraceptive method.

Hussein O Ammar *et al*., 2009, developed polymeric matrix system for prolonged delivery of tramadol hydrochloride. This study focused on bioadhesion, skin tolerability, and pharmacodynamic evaluation. The results showed that the polymeric systems appear to be an attracting way enabling the tailoring of the intended formula.

Ashu Mittal *et al.,* 2009, formulated and evaluated monolithic matrix polymer films for transdermal delivery of Nitrendipine. In this study the polymeric films of nitrendipine were prepared by the film casting technique on mercury substrate. They were evaluated for physicochemical parameters invitro release and exvivo permeation. From the result they concluded that release of the drug from the films followed anomalous transport ($0.5 < n < 1$).

Gattani S.G *et al.,* 2008, optimized transdermal films of lovastatin. In this study, monolithic matrix typed transdermal films of lovastatin were prepared by film casting technique on mercury substrate. All the formulations containing 10 % W/W of lovastatin and 30 % W/W of Dibutyl phalate in chloroform. From this study, they concluded that the polymeric matrix type transdermal films of lovastatin prepared with different grades and ratios of polymers holds potential for transdermal delivery.

Chandra Amrish *et. al*., 2008, developed transdermal delivery of ketorolac. In this work, studies were carried out to investigate the effect of permeation enhancers on the invitro permeation of Ketorolac across rat skin. They concluded that a reservoir type transdermal patch for delivery of Ketorolac thus appear to be feasible of delivering Ketorolac across skin.

Audra L. Stinchcomb *et al*., 2008, developed in vivo evaluation of a transdermal co drug of 6-β-naltrexol linked to hydroxybupropion in hairless quinea pigs. This study was carried out in order to determine percutaneous absorption of a transdermal co drug of naltrexol, 6-β-naltrexol hydroxybupropin co drug in hairless quinea pigs as well as to evaluate the safety of 6-β-naltrexol for development as a transdermal dosage form. The result of this study showed that a transdermal co drug of 6-β-naltrexol could be a viable alternative treatment for alcohol & opiate abuse.

Aisha Khanum *et al*., 2008, prepared and evaluated of tolterodine tartarate transdermal films for the treatment of overactive bladder. In this study a number of polymers such as hydroxy propyl methyl cellulose, carbopol-934P, and ethylcellulose were employed alone and an combination for the preparation of transdermal films were then evaluated for various physicochemical properties like physical appearance, weight variation, thickness, drug content, folding endurance and % elongation including in vitro release study. The result of this study shows that the combination of HPMC: Carbopol - 934P (3: 1) with 30 % PG films were very flexible with high folding endurance and uniform drug content.

Garrigues T.M *et al*., 2007, Analyzed nortriptyline hydrochloride skin absorptions and development of a transdermal patch. In this study the influence of propylene glycol, ethanol and oleic acid on nortriptyline hydrochloride penetration through human epidermis was studied in vitro at two different pH values (5.5 and 7.4). The results of this work showed that nortriptyline

hydrochloride permeates through the skin by passive diffusion; pH influence is remarkable for this molecule.

Samir Mitragatri *et al*., 2007, analyzed synergistic effects of chemical enhancers on skin permeability. They report on the transport enhancing properties of mixture of an anionic surfactant (sodium lauroyl sarcosinate) and a non ionic surfactant (sorbitan monolaurate S (20)) in PBS: ethanol (1:1) solvents. Results show that increased aggregation showed resemblance to these that exhibited highest skin permeabilization.

Luo Jia – bo *et al.,* 2007, studied effects of Cinnamene enhancers on transdermal delivery of ligustrazine hydrochloride. In this study the effects and mechanisms of penetration promoters on in vitro percutaneous absorption of ligustrazine hydrochloride across hairless porcine dorsal skin were investigated, transdermal fluxes of ligustrazine hydrochloride through porcine skin were determined in vitro by Franz – type diffusion cells. The results showed that the permeation enhancement mechanisms of cinnamene are disordering the lipids, extracting the lipids and competitive hydrogen bonding between cinnamene enhancers and amides of ceramide head groups in stratum corneum.

Sang Chul Shin *et al.,* 2006, evaluated the physicochemical characteristics of quinupramine in the EVA matrix. In this study an attempt to determine the state of drug in the ethylvinyl acetate (EVA) matrix, X-ray diffraction FTIR and thermal analysis of the quinupramine EVA matrix were carried out and the results were compared with those of a physical mixture of quinupramine and EVA at the same ratio. They concluded that the physicochemical interactions between quinupramine and EVA might occur at the molecular level and that quniupramine was not crystalline in the EVA matrix.

Patrizia Santi *et al*., 2006, formulated single-layer transdermal film containing lidocaine. The aim of this work was to modulate the delivery of the model drug lidocaine hydrochloride from the transdermal film across rabbit ear skin. They evaluate the effect of drug loading of film-forming polymer type, content of adhesive and plasticizer on lidocaine transport across the skin. They concluded that film forming polymer molecular weight had a negligible effect on drug permeation while its content was more effective and the choice of the adhesive seems to be the most important variable governing drug transport.

Santi P *et al*., 2006, evaluated new transdermal bioadhesive film containing oxybutynin and studied Invitro permeation across rabbit ear skin. The results obtained in this study showed that the bioadhesive film can be a promising and innovative therapeutic system for the transdermal administration of oxybutynin. In this study the film was applied in occlusive conditions the release profiles were much higher than in non-occlusive conditions, reaching 50 % of drug peremeated after 24 hr compared to the commercial patch oxytrol, the film was more efficient suggesting that a smaller area or a lower drug loading could be employed.

Mohd Aqil *et al*., 2006, evaluated In vivo characterization of monolithic matrix type transdermal drug delivery systems of pinacidil monohydrate. The aim of this study was to characterize transdermal drug delivery systems of pinacidil monohydrate in vivo by monitoring the effect of the transdermal drug delivery system on blood pressure of methyl prednisolone actate induced hypertensive rats. From this study they concluded that a single patch application of pinacidil transdermal drug delivery system can effectively control hypertension in rats for 2 days.

Srinivas *et al*., 2005, formulated and developed invitro and in vivo evaluation of membrane controlled transdermal systems of glibenclamide. In this study transdermal systems were prepared using drug containing carbopol gel as reservoir and ethyl cellulose, EudragitRS100, EudragitRL100 and Ethyl vinyl acetate. The formulations were subjected to various physicochemical studies, invitro drug release studies and permeation studies through mouse skin. Variation in drug release, permeation profile among the formulations containing different rate controlling membranes was observed. The present study shows that membrane controlled transdermal systems of glibenclamide exhibited better control of hypoglycemia and more effectively reversed the diabetes mellitus complications than oral glibenclamide administration in mice.

Taravat Ghafourian *et al.,* 2004, analyzed the effect of penetration enhancer on drug delivery through skin, a QSAR study. In this study, the structural requirements of penetration enhancers have been investigated using quantitative structure activity relationship (QSAR) technique. The resulting QSARS for enhancement towards different drugs incorporated different structural descriptors, suggesting the involvement of different mechanisms. For 5-fluorouracil & disodium, molecular descriptors in the corresponding QSARs indicated the possible involvement of intermolecular, electron donor, acceptor interactions.

Charles M. Heard *et al.,* 2004, developed in vitro transdermal delivery of the major catechins & caffeine from extract of camellia sinensis. The aim of this study was to investigate the feasibility of the transdermal delivery of catechins & caffeine from green tea extract. Transdermal delivery was determined across full thickness pig ear skin from saturated solutions of green tea extract in pH 5.5 citrophosphate buffer, PEG 400 & 50:50 mixture of citrate phosphate buffer & PEG in addition to patch containing 1.35mg/cm^2. The result indicates that the permeation from the patch

was within the range of C_{max} plasma levels achieved after oral dosing of 2.2-4.2 gm^{-2} green tea extract.

Ramesh Panchanula *et al.*, 2002, developed transdermal delivery of zidovudine and determined the effect of vehicles on permeation across rat skin and their mechanism of action. The purpose of this study was to investigate the effects of various solvent systems containing water, ethanol, PG and their binary combinations on the exvivo permeation of zidovudine across spraque dawlay rat skin using Franz diffusion cells at 37^0C. From the result they concluded that highest flux and short lag time were achieved with ethanol at 66.6% in water and hence it's a suitable vehicle for transdermal delivery of zidovudine.

Hassan Arabi *et al.*, 2002, prepared transdermal delivery system and evaluated the effect of membrane type for scopolamine drug. In this study scopolamine hydrobromide was incorporated into 2 polymers and release rate across ethylene-vinyl acetate copolymers & EC membranes were measured. The results showed that the rate of release increases with the increase of porosity size of EC surface despite of having high molecular weight.

Stanislaw Janicki *et al.*, 2001, developed matrix-type transdermal systems and evaluated the penetration of terpenes from the transdermal systems through human skin by Invitro studies. In this study polyurethane matrices containing upto 39 % of the terpenes, eucalyptol, L-limonene, D-limonene, dipentene or terpinolene were produced. From the result they concluded that penetration of terpenes was slower in the presence of epidermis, release and penetration through the epidermis and dermis were fastest for dipentene, being at least 3-4 times faster than for D-limonene and L-limonene.

Guyot M *et al.,* 2000, developed design and invitro evaluation of adhesive matrix for transdermal delivery of propanolol. In this study the influence of different factors was investigated. The result showed that in all matrices types, propylene glycol accelerated propranolol release rate.

Belal F *et al*., 2000, developed spectrophotometric determination of benazapril in tablets. In this study a simple and sensitive spectrophotometric method has been developed for the determination of benazepril hydrochloride in pharmaceutical formulations. The proposed method could be applied to the determination of benazepril in presence of the co formulated drug, hydrochlorthiazide.

Douw G. Muller *et al*., 2000, Performed a comparative study of an in situ adapted diffusion cell and an in vitro Franz diffusion cell method for transdermal absorption of doxylamine. The aim of this study was to compare the invitro Franz diffusion cell method with an in situ adapted diffusion cell method. The results showed that excised skin undergoes sub-lethal injury (necrosis) during in vitro experiments, which may lead to increased permeability of the drug. Considering these advantages and the results obtained from this study, they concluded that the in vitro Franz diffusion cell is the method of choice for experiments on percutaneous absorption but care must be taken when performing studies over extended periods, since it was shown that the degradation of excised skin occurs.

Riitta Sutinen *et al.,* 1999, Analyzed water activated pH controlled patch in transdermal administration of timolol. The aim of this study was to test the suitability of the patch design in transdermal delivery & further to select such transdermal patch formulations to a clinical study with timolol. The effect of skin on drug release was evaluated in vitro with this devices both the

rate of drug release & the duration of constant release were controlled. On the basis of in vitro data & kinetic simulations, devices of $10cm^2$ volume releasing timolol in vitro at the rates of $10\mu g\ h^{-1}\ cm^2$ were selected for human tests.

Mura P *et al.,* 1999, evaluated transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations. In this study the influence of diethyleneglycol monoethyl ether (transcutol) alone or in combination with PG, on clonazepam permeation through an artificial membrane and excised rabbit ear skin from carbopol hydro gels was investigated, the result is explained on the basis of the particular mechanism of action demonstrated for transcutol which associates the increase of drug solubility to the potent effect of a depot in the skin.

Minghetti P *et al.,* 2000, developed local patches containing melilot extract. Two types of methacrylate patches were prepared. The data of the exvivo coumarin skin permeation and those obtained by the in vivo stripping technique showed a good correlation. The coumarin permeated across the skin in vivo correlated well with those permeated exvivo, therefore exvivo permeation test can be useful to predict the amount of coumarin absorbed in vivo.

Charles M. Heard *et al.,* 1999, evaluated the therapeutic dose of primaquine can be delivered across excised human skin from simple transdermal patches. This work investigated the permeation of primaquine across full thickness excised human skin from two acrylate transdermal adhesives. From this study they determined that a simple patch with a diameter of $\approx 13\ cm^2$ could deliver a therapeutic in vivo dose, with possibilities for the treatment and prophylaxis of malaria and also concluded that the presence of migliol 840 failed to produce the anticipated enhancing effect.

Agrawal S.S *et al.,* 1996, formulated transdermal controlled administration of verapamil enhancement of skin permeability. In this study transdermal drug delivery systems of verapamil hydrochloride using hydrophilic polymers PVA & PVP and different concentrations of enhancers, d-limonene was developed. Result obtained showed that permeation rate was enhanced & followed approximately zero order kinetics.

Fusun Acarture *et al*., 1995, investigated the effect of different adjuvant on felodipine release kinetics from sustained release monolithic films. The sustained release monolithic films are developed by employing a calcium channel blocker, felodipine, with two acrylic resin polymers of varying permeability. The relationship between the invitro drug release data, moisture permeation constant and glass transition temperature was investigated. They concluded that in vitro release rate of drug increased with increasing water vapor transmission and no relationship was established between glass transition temperature of the films and invitro release of drug.

Suresh P. *Vyas et al*., 1989, developed effective and controlled transdermal delivery of ephedrine. In this study the drug plasma profiles were compared with the plasma profile obtained following the administration of normal oral multiple doses of ephedrine hydrochloride using conventional tablets. After transdermal application of half the doses of ephedrine, as compared with the conventional dose recommended for administration during 24 hours, constant and comparatively higher drug blood level could be achieved. The most promising in vivo availability of the drug was recorded with selected pseudolatices.

Rosilio V *et al.,* 1988, Evaluated physico-chemical characterization of EC drug loaded cast films. In this study the influence of solvent on certain physico chemical properties cast unloaded

& SIBA loaded EC films has been studied using chloroform, ethanol and a mixture of chloroform and ethanol. The result obtained in this study have shown that the wetting properties and permeability of the films significantly affected by solvents. They concluded that the effect of solvent is particularly significant in the case of films cast from chloroform, ethanol mixture.

Srini N. Tenjarala *et al*., investigated synthesis and evaluation of N-acetyl prolinate esters novel skin penetration enhancers. In this study a series of N-acetyl prolinate esters (alkyl side chain length, 5-18) were synthesized and tested for potential skin penetration enhancement activity using modified Franz diffusion cells and hairless moused skin as the penetration barrier. The result showed that maximum flux increase was obtained with the 11 and 12 carbon (alkyl chain length) esters for both benazepril and hydrocortisone. The 18-carbon ester which has a cis-double bond in the alkyl side chain also increased the flux significantly.

Anilreddy B. *et al.,* Analyzed invitro characterization and evaluation of transdermal drug delivery system for metoprolol tartarate transdermal films of metoprolol tartarate were prepared using polymers such as EC, PVA, ER L100, EL 100, DBP was used as plasticizer. In vitro drug release kinetics was studied using Franz diffusion cell drug release followed zero order kinetics. In conclusion combination of EC, PVA, ER L100, and EL100 & DBP can potentially be optimized to develop an effective transdermal drug delivery system for metroprolol tartarate.

CHAPTER IV

AIM AND OBJECTIVE

The transdermal route of administration has been recognized as one of the highly potential routes. Transdermal drug delivery is the delivery of drugs across epidermis to achieve systemic effects. Transdermal patches control the delivery of drugs at controlled rates by employing an appropriate polymer. This route allows controlled release of the drug at rates approaching zero-order simulating those provided by intravenous infusion.

The skin is one of the most extensive and readily accessible organs of the human body. It receives about one-third of the blood circulation through the body. Hence the skin has been explored as the port of entry of drugs.

Development of transdermal drug delivery system offers a possible approach to overcome some of the drawback of oral therapy such as,

a) This route improves compliance of the patient

b) Ensures essentially constant drug input

c) Bypasses the gastrointestinal tract and the liver as sites of metabolism, that are responsible for the low oral bioavailability of drugs.

Benazepril hydrochloride is an angiotension converting enzyme inhibitor used to treat hypertension, heart failure, to reduce proteinuria, renal disease in patient with nephropathies, prevent stroke, myocardial infarction and cardiac death in high risk patients. The extent of absorption is about 37%, it undergoes extensive first pass metabolism and has a short biological

half life (3 hours). It could be a promising candidate in transdermal system design taking into accounts its high lipid solubility and penetration behavior.

The aim of this study is to develop suitable transdermal patches of benazepril hydrochloride by employing ethylcellulose, Eudragit S100 and Eudragit L100 as a film former and to investigate the effect of polymers, plasticizer and permeation enhancer on *in vitro* release of transdermal patches of benazepril hydrochloride.

CHAPTER V

PLAN OF WORK

The plan of work involves formulation and evaluation of matrix type transdermal patches of benazepril hydrochloride.

PART A

PREPARATION OF STANDARD CALIBRATION CURVE

a) Preparation of dissolution medium - Phosphate buffered saline pH 7.4

b) Preparation of calibration curve for benazepril hydrochloride

PART B

DRUG – POLYMER INTERACTION STUDIES

a) FTIR study

b) Differential Scanning Calorimetry Study

PART C

FORMULATION OF BENAZEPRIL HCL TRANSDERMAL PATCHES

a) Formulation of transdermal patches of Benazepril Hydrochloride using solvent casting technique.

PART D

EVALUATION OF TRANSDERMAL PATCHES

a) Physical appearance

b) Weight variation

c) Thickness of the films

d) Folding endurance

e) Flatness

f) Percentage moisture content

g) Estimation of drug content

h) Invitro drug release studies

i) Study of drug release kinetics

j) Ex-vivo permeation studies

k) Statistical analysis

l) Histopathology studies

PART E

SCANNING ELECTRON MICROSCOPY

a) The surface morphology of the patch before and after exvivo permeation study using scanning electron microscopy.

CHAPTER VI

MATERIALS AND EQUIPMENTS

MATERIALS

1. Benazepril Hydrochloride	-	Gift sample from Safe Tab Life Science, Puducherry.
2. Ethyl cellulose	-	Gift sample from Safe tab Life Science, Puducherry.
3. Eudragit L100	-	Gift sample from Orchid Pharmaceuticals, Chennai.
4. Eudragit S100	-	Gift sample from Orchid Pharmaceuticals, Chennai.
5. Dibutyl phthalate	-	Loba Chemis private limited, India.
6. Poly ethylene glycol	-	Bargoyne urbidges & Co, Mumbai.
7. Dimethyl sulfoxide	-	The British Drug Houses Ltd, England.
8. Ethanol	-	Changshu Yangyuah chemicals, China.
9. Potassium dihydrogen phosphate	-	High purity laboratory chemicals, Mumbai.
10. Disodium hydrogen phosphate	-	Nice chemicals Pvt Ltd, Kerala.

11. Sodium chloride	-	Central Drug House (P) Ltd, New Delhi.
12. Formaldehyde solution	-	Astron chemicals, Ahmedabad.
13. Surgical spirit	-	Tansi polish unit, Madras..

EQUIPMENTS

1. Petri plate	-	Universal Scientifics, Madurai.
2. Electronic weighing balance	-	A & D Company, Japan.
3. Hot air oven	-	RIC Scientific Research & laboratory Instruments, Chennai.
4. Vernier caliper	-	Linker, India
5. Dissolution apparatus (Lab India DS 8000)	-	Lab India Instruments Pvt Ltd. Navi mumbai.
6. Franz diffusion cell	-	Universal Scientifics, Madurai.
7. Magnetic stirrer with hot plate	-	M.C. Dalal & Co, Chennai.
8. UV-Visible spectrophotometer	-	Shimadzu Corporation, Japan.
9. Micro slides	-	M.C.Dalal &Co, Chennai.
10. Scanning electron microscope	-	Hitachi S-3400, Japan.

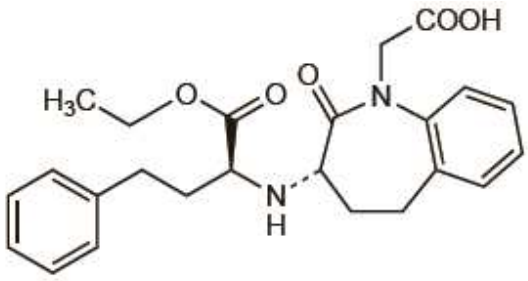
11. FT-IR	-	Shimadzu, Japan.
12. Differential Scanning Calorimetry	-	DSC Q 200, Mumbai.
13. Refrigerator	-	Kelvinator, India.

CHAPTER VII

DRUG PROFILE

BENAZEPRIL HYDROCHLORIDE

STRUCTURAL FORMULA



SYNONYMS:

Benazepril HCl

Benazepril Hydrochloride

Benazeprilum (Latin) (Drug Bank: Benazepril).

EMPIRICAL FORMULA:

$C_{24}H_{28}N_2O_5$, HCl

CHEMICAL NAME :

{(3S)-3-[(1S)-1-Ethoxycarbonyl-3-phenylpropylamino]-2,3,4,5-tetrahydro- 2-oxo-1H-1-benzazepin-1-yl}acetic acid hydrochloride; 1-Carboxymethyl-3-[1-ethoxycarbonyl-3-phenyl-(1S)-propylamino]-2,3,4,5-tetrahydro-1H-1(3S) benzazepin-2-one hydrochloride.

DESCRIPTION:

Nature	:	White crystalline powder
Solubility	:	Soluble in water, ethanol and methanol
Melting point	:	148.5º
Molecular weight	:	424.49 g/mol
pKa	:	5.3
log P	:	3.3

CHEMICAL PROPERTIES:

Loss on drying	:	Not more than 0.5 % W/W
Residue on ignition	:	Not more than 0.1 % W/W
Heavy metals	:	Not more than 0.001 %

MECHANISM OF ACTION:

Benazepril and benazeprilate inhibit angiotensin-converting enzyme (ACE) in human subjects and animals. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex.

Inhibition of ACE results in decreased plasma angiotensin II, which leads to decreased vasopressor activity and to decreased aldosterone secretion. The latter decrease may result in a small increase of serum potassium.

While the mechanism through which benazepril lowers blood pressure is believed to be primarily suppression of the renin-angiotensin-aldosterone system, benazepril has an antihypertensive effect even in patients with low-rennin hypertension (Drug Bank: Benazepril).

PHARMACOKINETICS:

Absorption:

Peak in plasma within 0.5-1.0 Hours. The extent of absorption is atleast 37% as determined by urinary recovery and is not significantly influenced by the presence of food in the GI tract.

Distribution in blood:

Benazeprilate is not extensively distributed into extravascular sites with minimum passage across the blood/brain barrier.

Metabolism:

Cleavage of the ester group (primarily in the liver) converts benazepril to its active metabolite, benazeprilate. Benazepril and benazeprilat may be conjugated to glucuronic acid prior to urinary excretion.

Excretion:

Benazepril and benazeprilate are cleared predominantly by renal excretion in healthy subjects with normal renal function. Nonrenal (i.e., biliary) excretion accounts for approximately 11%-12% of benazeprilat excretion in healthy subjects (Colin Dollery., 1999, Drug Bank: Benazepril).

INDICATIONS AND USAGE:

Control of arterial hypertension

Treatment of congestive heart failure (Colin Dollery., 1999)

DOSE:

An initial dose of 10 mg once daily is recommended in patients with creatinine clearance ≥30 ml /min and those not receiving diruetics.

Hypertensive patients with heart failure and those with a creatinine clearance ≥ 30 ml /min are recommended an initial daily dose of 5 mg (Anthony C Moffat., 2004, Colin Dollery., 1999).

ADVERSE EFFECTS

Serious Reactions

- angioedema, head/neck
- angioedema, intestinal
- hypotension, severe
- hyperkalemia
- renal impairment/failure
- hepatotoxicity
- neutropenia
- agranulocytosis
- anemia, hemolytic
- thrombocytopenia
- pancreatitis
- Stevens-Johnson syndrome
- pemphigus
- oligohydramnios (in utero exposure)
- fetal/neonatal harm or death (in utero exposure)
- congenital malformations, major (1st trimester use)

Common Reactions

- cough
- hypotension

- dizziness
- fatigue
- hyperkalemia
- nausea/vomiting
- elevated Cr
- musculoskeletal pain
- photosensitivity

DRUG INTERACTIONS:

Potentially hazardous interactions:

Increased hypotensive effects occur when benazepril is combined with thiazide diuretics or dihydropyrimidine calcium antagonist (Colin Dollery., 1999).

CONTRAINDICATIONS:

Angioedema

Pregnancy (Colin Dollery., 1999).

STORAGE:

Preserve in well closed container store below 30º C.

CHAPTER VIII

EXCIPIENTS PROFILE

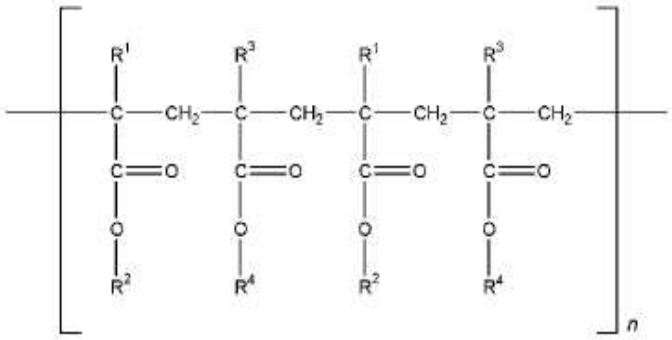
POLYMETHACRYLAT (EUDRAGIT L100)

Synonyms	:	Acryl-EZE; Acryl-EZE MP; Eastacryl 30D; Eudragit KollicoatMAE 30 D; Kollicoat MAE 30 DP; Polymeric methacrylates.
Nonproprietary names	:	BP: Methacrylic acid–ethyl acrylate copolymer (1: 1) PhEur: Acidum methacrylicum et ethylis acrylas polymerisatum (1: 1) Acidum methacrylicum et ethylis acrylas polymerisatum (1: 1) dispersio 30 per centum Acidum methacrylicum et methylis methacrylas (1: 1) Acidum methacrylicum et methylis methacrylas Polymerisatum (1: 2) Copolymerum methacrylatis butylati basicum Polyacrylatis dispersion 30 per centum USPNF: Ammonio methacrylate copolymer Methacrylic acid copolymer d copolymer dispersion

Chemical name : Poly (methacrylic acid, methyl methacrylate) 1 : 1

Empirical formula : $(C_5 H_8 O_2)_n$

Structural formula :



R^1, R^3, R^4 = CH3

R^2 = H

Description

- ❖ Nature : White free flowing powder
- ❖ Solubility : Soluble in acetone and alcohol
- ❖ Molecular weight : ≥ 100 000

Functional categories : Film former

Tablet binder

Tablet diluent

Properties		
Loss on drying	:	≤ 5.0%
Methyl methacrylate and methacrylic acid	:	≤ 0.1%
Sulfated ash	:	≤ 0.1%
Apparent viscosity	:	50–200 mPa s
Stability and storage	:	Dry powders are stable for at least 3 years if stored in a tightly closed container at less than 30ºC.
Assay	:	Methacrylic acid units 46.0–50.6%

(Raymond C. Rowe *et al*., 2006).

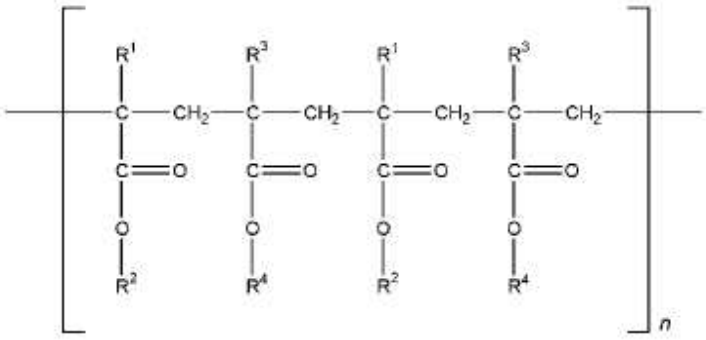
POLYMETHACRYLAT (EUDRAGIT S100)

Synonyms	:	Acryl-EZE; Acryl-EZE MP; Eastacryl 30D; Eudragit; KollicoatMAE 30 D; Kollicoat MAE 30 DP; polymeric methacrylates.
Nonproprietary names	:	BP: Methacrylic acid–ethyl acrylate copolymer (1 : 1) PhEur: Acidum methacrylicum et ethylis acrylas polymerisatum (1 : 1) Acidum methacrylicum et ethylis acrylas polymerisatum (1 : 1) dispersio 30 per centum Acidum methacrylicum et methylis methacrylas Polymerisatum (1 : 1) Acidum methacrylicum et methylis methacrylas Polymerisatum (1 : 2) Copolymerum methacrylatis butylati basicum Polyacrylatis dispersion 30 per centum USPNF: Ammonio methacrylate copolymer Methacrylic acid copolymer Methacrylic acid copolymer dispersion

Chemical name : Poly (methacrylic acid, methyl methacrylate) 1 : 2

Empirical formula : $(C_5 H_8 O_2)_n$

Structural formula



$R^1, R^3, R^4 = CH3$

$R^2 = H$

Description

- ❖ Nature : White free flowing powder
- ❖ Solubility : Soluble in acetone and alcohol
- ❖ Molecular weight : ≥ 100 000

Functional categories : Film former

Tablet binder

Tablet diluents

Properties		
Loss on drying	:	≤ 5.0%
Methyl methacrylate and methacrylic acid	:	≤ 0.1%
Sulfated ash	:	≤ 0.1%
Apparent viscosity	:	50–200 mPa s
Stablility and storage	:	Dry powders are stable for at least 3 years if stored in a tightly closed container at less than 30ºC.
Assay	:	Methacrylic acid units 27.6–30.7%

(Raymond C. Rowe *et al*., 2006).

ETHYL CELLULOSE

Synonyms : Aquacoat ECD; Aqualon; E462; Ethocel; Surelease.

Nonproprietary names : BP: Ethylcellulose

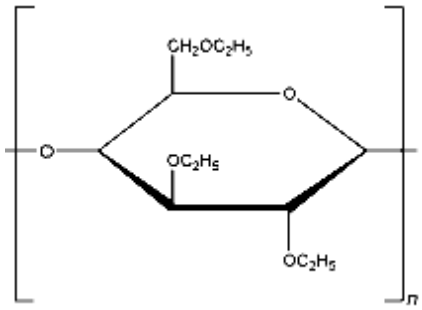
PhEur: Ethylcellulosum

USPNF: Ethylcellulose

Chemical name : Cellulose ethyl ether

Empirical formula : $C_{12}H_{23}O_6\,(C_{12}H_{22}O_5)n\,C_{12}H_{23}O_5$

Structural formula



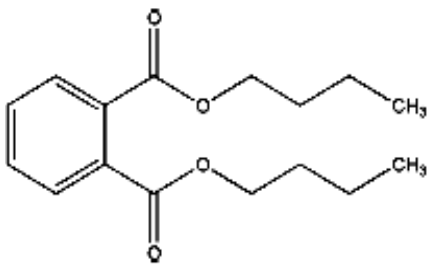
Description : Ethylcellulose is a tasteless, free-flowing, white to light tan colored powder.

Functional categories	:	Coating agent; flavoring fixative; tablet binder; tablet filler; viscosity- increasing agent.
Properties		
Loss on drying	:	≤ 3.0%
Residue on ignition	:	≤ 4.0%
Ethoxyl groups	:	44.0–51.0%
Melting point	:	165^0C to 180^0 C
Solubility	:	Ethylcellulose is practically insoluble in glycerin, propylene glycol, and water. Ethylcellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol (95%).
Specific gravity	:	1.12–1.15 g/cm^3
Nominal viscosity	:	6–10 mPa s
Stablility and storage	:	Ethylcellulose is a stable, slightly hygroscopic material. Ethylcellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures (Raymond C. Rowe *et al*., 2006).

DIBUTYL PTHALATE

Synonyms	:	Araldite 502; benzenedicarboxylic acid; benzene-o-dicarboxylic acid di-n-butyl ester; butyl phthalate; Celluflex DBP; Genoplast B; Hatcol DBP; Hexaplast M/B.
Nonproprietary names	:	BP: Dibutyl Phthalate PhEur: Dibutylis phthalas
Chemical name	:	Dibutyl benzene-1,2-dicarboxylate
Empirical formula	:	$C_{16}H_{22}O_4$
Molecular weight	:	278.34

Structural formula

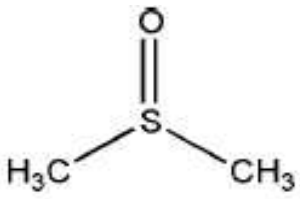


Description	:	Dibutyl phthalate occurs as an odorless, oily, colorless, or very slightly yellow-colored, viscous liquid.
Functional categories	:	Film-former; plasticizer; solvent.
Properties		
Flash point	:	171^0c
Boiling point	:	340^0c
Refractive index	:	1.491 to 1.495
Solubility	:	very soluble in acetone, benzene, ethanol (95%), and Ether; soluble 1 in 2500 of water at 20^0C.
Relative density	:	1.043–1.048
Dynamic viscosity	:	20 mPa s
Stability and storage	:	Dibutyl phthalate should be stored in a well-closed container in a cool, dry, location. Containers may be hazardous when empty since they can contain product residues such as vapors and liquids (Raymond C. Rowe *et al*., 2006).

DIMETHYL SULFOXIDE

Synonyms	:	Deltan; dimexide; dimethyl sulphoxide; DMSO; Kemsol; Methylsulfoxide; Rimso-50; sulphinylbismethane (Raymond C. Rowe *et al*., 2006).
Nonproprietary names	:	BP: Dimethyl sulfoxide PhEur: Dimethylis sulfoxidum USP: Dimethyl sulfoxide.
Chemical name	:	Sulfinylbismethane
Empirical formula	:	C_2H_6OS
Molecular weight	:	78.13

Structural formula

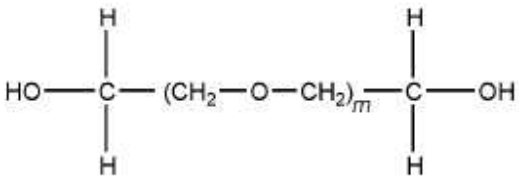


Description	:	Dimethyl sulfoxide occurs as a colorless, viscous liquid, that are miscible with water, alcohol, and ether, slightly bitter taste with a sweet aftertaste and has a slight odor characteristic of dimethyl sulfoxide.
Functional categories	:	Penetration enhancer; solvent.
Properties		
Flash point	:	95^0c
Boiling point	:	189^0c
Refractive index	:	1.478–1.479 (PhEur 2005)
1.4755–1.4775 (USP 28)		
Solubility	:	Miscible with water with evolution of heat; also miscible with ethanol (95%), ether and most organic solvents; immiscible with paraffins, hydrocarbons. Practically insoluble in acetone, chloroform, ethanol (95%), and ether.
Relative density	:	1.043–1.048
Dynamic viscosity	:	1.1 mPa s (1.1 cP) at 27^0C
Stability and storage	:	Dimethyl sulfoxide is reasonably stable to heat but upon prolonged reflux it decomposes slightly to methyl mercaptan and bismethylthiomethane. Dimethyl sulfoxide should be stored in airtight, light resistant containers.

POLYETHYLENE GLYCOL

Synonyms	:	Carbowax; Carbowax Sentry; Lipoxol; Lutrol E; PEG; Pluriol E; polyoxyethylene glycol.
Nonproprietary names	:	BP: Macrogols JP: Macrogol 400 PhEur: Macrogola USPNF: Polyethylene glycol
Chemical name	:	a-Hydro-o-hydroxypoly (oxy-1,2-ethanediyl)
Empirical formula	:	$HOCH_2 (CH_2OCH_2)_m CH_2OH$
Molecular weight	:	380–420

Structural formula



Description	:	Liquid grades (PEG 200–600) occur as clear, colorless or slightly yellow-colored, viscous liquids. They have a slight but characteristic odor and a bitter, slightly burning taste.
Functional categories	:	Ointment base; plasticizer; solvent; suppository base; tablet and capsule lubricant.
Properties		
Flash point	:	238^0c
Refractive index	:	1.465
Solubility	:	Polyethylene glycols are soluble in acetone, alcohols, benzene, glycerin, and glycols.
Relative density	:	1.11–1.14 g/cm^3
Dynamic viscosity	:	105–130 mPa s
Stability and storage	:	Polyethylene glycols are chemically stable in air and in solution. Polyethylene glycols should be stored in well closed containers in a cool, dry place. Stainless steel, aluminum, glass, or lined steel containers are preferred for the storage of liquid grades. (Raymond C. Rowe *et al*., 2006).

EXPERIMENTAL DETAILS

A. PREPARATION OF STANDARD CALIBRATION CURVE

Preparation of dissolution medium - phosphate buffered saline p^H 7.4:

Phosphate buffered saline p^H is prepared by dissolving 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen ortho phosphate and 8.0 g of sodium chloride in sufficient amount of water to produce 1000 ml (I.P 1996).

Preparation of calibration curve for benazepril hydrochloride:

For the preparation of calibration curve 100 mg benazepril hydrochloride is weighed and dissolved in a small quantity of Phosphate buffered saline p^H 7.4 in a 100 ml standard flask and made up to the volume with Phosphate buffered saline p^H 7.4. From this primary stock solution 10 ml is pipetted out and made up to 100 ml with Phosphate buffered saline p^H 7.4 to form the secondary stock solution resulting in the concentration of 100 µg/ml. From the secondary stock solution 2ml, 4ml, 6ml, 8ml, 10ml, 12ml, 14ml 16ml, 18ml, 20ml samples are pipetted into 100 ml volumetric standard flasks separately and made up to the volume with Phosphate buffered saline PH 7.4 to get concentrations of 2 µg/ml, 4 µg/ml,6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml, 14 µg/ml, 16 µg/ml, 18 µg/ml, 20 µg/ml of drug solutions respectively (Beata Stanisz *et al*., 2009).

λ_{max} is found by scanning the benazepril hydrochloride solution under UV-Visible spectrophotometer. The absorbance is measured at λ_{max} for different concentrated solutions to

obtain standard calibration curve. Standard calibration curve is plotted by taking concentration in x-axis and absorbance in y-axis.

B. DRUG – POLYMER INTERACTION STUDIES

This is carried out to check the compatibility between drug and various polymers. It is therefore necessary to confirm that drug is not interacting with polymers under experimental conditions and throughout the shelf life (Meenakshi Bharkatiya *et al*., 2010). The physicochemical compatibility between the drugs and polymers used in patches is studied by using FTIR and DSC studies.

1) FTIR studies:

Infra red spectroscopy is carried out on pure drug and physical mixtures of drug: polymer at 1:1 ratio is carried out between 500cm^{-1} – 5000cm^{-1} (Meenakshi Bharkatiya *et al*., 2010).

2) Differential Scanning Calorimetry Study

The physicochemical compatibility between the drugs and polymers used in patches is studied by using DSC studies. The DSC of the pure drug and physical mixtures of drug: polymer at 1:1 ratio is carried out. The sample is heated between 50ºC and 250º at the rate of 10ºC/min in an atmosphere of nitrogen (20 ml/min). The thermograms obtained for the drug, polymers, physical mixture of drugs with polymers are compared (Shyan Sundar Agrawal *et al*., 2011).

C. FORMULATION OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCHES

Matrix type transdermal patches containing benazepril hydrochloride are prepared by solvent casting technique employing a mercury substrate (Ashu Mitlal *et al*., 2009). Polymer solutions are prepared using ethanol as solvent (Rosilio V *et al*., 2009). To the polymeric solution known weight of drug (benazepril hydrochloride 69.3 mg) is added and mixed slowly with a glass rod for 20 minutes until a homogenous drug polymer solution is formed (Meenakshi Bharkatiya *et al*., 2010). Then plasticizer and permeation enhancer of required quantity are added and mixed thoroughly (Jianping Liu *et al*., 2009). The resulting homogenous drug-polymeric solution is poured on a mercury substrate (area of 13.86 cm^2) in a petridish and dried at room temperature (Anilreddy B *et al*., 2010).The rate of evaporation of solvent is controlled by inverting a funnel over the petridish (Shashikant D. Barhate *et al*., 2009). The film formation is noted by observing the mercury surface after complete evaporation of the solvent (Meenakshi Bharkatiya *et al*., 2010). After drying at room temperature for 24 hours, membranes are taken out, packed in aluminium foil (Mamatha T., 2010) and stored in dessicator until further use (Anilreddy B *et al*., 2010).

D. EVALUATION OF TRANSDERMAL PATCHES

1) Physical appearance:

All the transdermal patches are visually evaluated for their physical appearance opaque/transparent, smooth/wrinkled, flexible/tough, and sticky/nonsticky (Shyam Sudar Agarwal *et al*., 2011)

2) Weight variation:

Weight variation is determined by weighing three patches individually, from each batch and the average weight, standard deviation are calculated for each formulation (Mamatha T *et al*., 2010).

3) Thickness of the patch:

The thicknesses of the transdermal patches are measured using a digital vernier caliper (Gilhotra Ritu Mehra *et al*., 2011). The measurements are made at three different places. The average and standard deviation of three readings are calculated for each formulation (Mamatha T *et al*., 2010).

4) Folding endurance:

Folding endurance is measured manually for the prepared patches. It is expressed as number of times the patch is folded at the same place either to break the patch or to develop visible cracks. This is important to check the ability of sample to withstand folding. This also gives an indication of brittleness. For the determination of Folding endurance a strip of film (2 cm^2) is cut evenly and repeatedly folded at the same place till it broken (Aisha Khanum *et al*., 2008). The number of times the patch can be folded at the same place without breaking/cracking give the value of folding endurance.

5) Flatness:

The constriction of patches cut from a drug loaded matrix patch is an indicator of its flatness. Longitudinal strips are cut out from the prepared medicated patch, the lengths of each

strip are measured and then variation in the lengths due to non – uniformity in flatness is measured. Flatness is calculated by measuring construction of strips and a zero percent constriction is equivalent to a hundred percent flatness (Priyanka Arora *et al*., 2002).

$$constriction\ (\%) = \frac{L1 - L2}{L1} \times 100$$

Where,

L_1 = Initial length of each strip,

L_2 = Finial length of each strip.

6) Percentage moisture content:

The prepared films are weighed individually and kept in a desiccator containing silica gel at room temperature for 24 hours. The films are again weighed and the percentage moisture content is calculated using the formula (Mamatha T *et al*., 2010).

$$\text{Percentage Moisture Content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \text{ X } 100$$

7) Estimation of drug content:

Transdermal patches of specified area (5 cm^2) are cut into small pieces and are transferred into 50 ml standard flask. About 5 ml of ethanol is added to dissolve the patch and then made upto 50 ml with phosphate buffer saline pH 7.4. The solution is filtered, from the filtrate 5ml is transferred into 50 ml standard flask and made upto 50ml with phosphate buffer

saline pH 7.4. The absorbance of the solution is measured at 241.5 nm using UV visible spectrophotometer (Divyesh Patel *et al*., 2011).

8) *Invitro* drug release studies:

The *in-vitro* drug release study for the transdermal patches are carried out using modified paddle over disc assembly (USP Apparatus 5) (Martin Siewert *et al*., 2003). The disc apparatus (European Pharmacopoeia 5.0) consists of mesh screen made of stainless steel clamped in the watch glass using nylon clips. The transdermal patch of specified area is pasted over a small piece of aluminium foil (backing layer) to prevent two dimensional release. The transdermal patch with backing layer is placed between inert stainless steel mesh and watch glass exposing the patch to the medium. It is also ensured that the patch does not float inside the disc assembly. The disc assembly containing transdermal patch is placed at the bottom of the dissolution vessel, with the mesh facing upwards, under the rotating paddle. The dissolution medium used is 900 ml of Phosphate buffered saline pH 7.4 (Yogesh M. Amgaokar *et al*., 2011). The apparatus is equilibrated to the temperature of 32 ± 0.5^0c operated at 50 ± 1 rpm. The dissolution study is carried out for 12 hours. 5 ml of samples are withdrawn at regular intervals of 15 minutes for 1 hour and then 30 minutes for next 11 hour. The same volume of corresponding dissolution medium is replenished to maintain sink condition. The amount of benazepril hydrochloride released is determined by measuring the absorbance of the samples at 241.5 nm using UV-Visible spectrophotometer. Each test is performed in triplicate.

9) Study of drug release kinetics

In order to investigate the drug release mechanism from patches, the percentage cumulative drug release data is analyzed with following mathematical model (Yogesh M. Amgao Kar *et al*., 2011)

Model	Equation
Zero order Kinetics	$Q = Q_0 - K_0 t$
First order kinetics	$Q = Q_0 (1 - e^{-K/t})$
Higuchi square root model	$Q_t = K_H t^{1/2}$
Korsmeyer-peppas model	$Q_t / Q_\infty = K_k t^n$
Hixson-Crowell cube root model	$\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = K_{HC}{}^t$

Where,

Q_t = Amount of drug released at time t

Q_0 = Initial amount of drug

K_0, K_1, K_H , K_{HC} and K_K are the coefficients of equation. The most appropriate model is selected on the basis of goodness of fit test. The zero order kinetic describes the systems in

which the drug release rate is independent of its concentration. The first order kinetics describes the system in which drug release rate is concentration dependent. Higuchi model describes the release of water – soluble drug from an insoluble matrix as a diffusion process based on the Fick's law and is square root time dependent. The Hixson – Crowell cube root law describes the drug release from a system depends upon the change in surface area or diameter of particle or system and involves no diffusion mechanism. Korsmeyer – Peppas model describes the fraction of drug release relates exponentially with respect to time. This model is generally used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved.

10) *Ex-vivo* permeation studies:

The *ex vivo* skin permeation experiments are conducted using vertical type Franz diffusion cells having receptor compartment capacity of 15 ml (Naohiro Nishida *et al*., 2010).

- **Preparation of rat skin membrane**

Permeation studies are carried out after obtaining ethical committee clearance (Ref. No. 14024/E1/4/2011). Wister strains of male albino rats weighing between 105-120 g (Anilreddy B et al., 2010) are used for this study. Membrane for the permeability studies is full thickness skin from the abdominal region of the rats. The hair present over the skin is removed by trimming and careful shaving so that the skin is not damaged. The skin is excised from rat after anaesthetizing. The epidermis is prepared surgically by heat separation technique, which involved soaking the entire abdominal skin in water at 60ºC for 45 sec, followed by careful

removal of the epidermis (Madhusudan Rao Y *et al.,* 2007). The excised skin samples are then stored in refrigerator at 0 - 4ºC and are used within three days (Yanli Gao *et al*., 2009).

❖ **Permeation studies**

The receptor compartment is filled with 15 ml of Phosphate buffered saline pH 7.4 (Young – Charg Ah *et al*., 2010). The transdermal patches with backing membrane are firmly pressed onto the centre of the rat skin. Once adhesion to the skin surface had been confirmed, the skin is quickly mounted on the diffusion cell receptor compartment such that the patch is tightly secured over the flange aperture. The donor compartment is then placed in position and the two halves of the cell are clamped together. The whole assembly is placed over a magnetic stirrer. The dissolution medium in the receptor compartment is stirred constantly using a magnetic bead. The samples of 0.5 ml are withdrawn at regular time intervals of 1 hour and analyzed for drug content. Receptor phases are replenished with equal volume of fresh receptor medium at each time interval (Shengjie Bian *et al*., 2003). Each permeation experiment is repeated three times. The cumulative amounts of drug permeated and corrected for acceptor sample replacement is plotted against time (Alireza Ghaffari *et al*., 2007).

❖ **Determination of permeation parameters**

For the determination of permeation parameter, the cumulative amount of drug permeated across the skin is plotted against time. The steady state flux (J) is calculated from the slope of the linear region of the above plot. The lag time (T) is calculated by extrapolating the linear region of the curve to the X – axis (Srini N. Tenjarla *et al*., 1999). The permeability coefficient (K_p) is calculated from the ratio of flux to drug concentration in the donor chamber.

$$K_p = J/C$$

Where,

K_p = Permeability coefficient

J = Flux

C = Initial drug load

11) Statistical analysis

Graph Pad In Stat Version 3.0 Software is used for statistical analysis. The cumulative amount permeated and flux values obtained are tested for the determination of significant differences using a one-way analysis of variance (ANOVA) (Venkateswara Rao J *et al*., 2010).

12) Histopathology studies:

Due to the possibility of changes to excised skin during extended *in vitro* experiments, Histopathological study is used to determine possible anatomical changes in rat skin. During the *invitro* experiments, a portion of skin (1 cm^2) is collected from the proximal and distal dorsal side of each mouse. The proximal dorsal skin is marked A, and immediately stored in 10 % buffered formalin solution. The distal dorsal skin is marked B and kept in phosphate buffered saline pH 7.4 at 32º C for a 12 hour period after which is stored in a 10 % buffered formalin solution. The skin portions in contact with transdermal patches and pure drug solution is removed from the Franz diffusion cells after the 12 hour experimental period and a cm^2disc of skin cut from the centre of each skin portion. The skin portion in contact with transdermal patch

containing Eudragit L100 with DMSO is marked D, Eudragit L100 without DMSO is marked E, and in contact with pure drug solution is marked C. The marked skin portions are stored in 10 % buffered formalin solution. Sample A is used as control for normal living skin. Sample B act as a control to represent the in vitro experiment without the presence of benazepril hydrochloride (in the form of pure drug or transdermal patch). The skin samples are embedded in paraffin and 6µm sections are cut using a microtome. Hemotoxylin and eosin staining are performed (Douw G. Muller *et al*., 2001).

Morphological changes in the skin (especially in epidermal layers) after the permeation experiments are observed visually and classified on a scale of A – D (Gasem K.A.M. *et al*., 2010).

CLASS	OBSERVATIONS	INFERENCE
A	Morphology of the sample looks exactly similar to the control	Nontoxic
B	Morphology looks almost similar to the control	Slightly toxic
C	Morphology includes partial epidermal degradation with nuclei bleeding in to the dermal layers	Toxic
D	Morphology includes severe epidermal degradation with cell death	Severely Toxic

E. SCANNING ELECTRON MICROSCOPY

The external morphology of the transdermal patches is analyzed using scanning electron microscope. The samples placed on the stubs were coated finely with gold palladium alloy and examined under the microscope (Biswajit Mukherjee *et al*., 2005).

CHAPTER X

RESULTS AND DISCUSSION

A. PREPARATION OF STANDARD CALIBRATION CURVE

Known concentration (10µg/ml) solution of benazepril hydrochloride in phosphate buffered saline pH 7.4 was scanned to find out the λ_{max} and it was found to be 241.5 nm. The result was shown in Figure 1. The calibration curve for benazepril hydrochloride was prepared in phosphate buffered saline pH 7.4 in the concentration range of 2 to 20 µg/ml. The absorbances were measured at λ_{max} of 241.5nm. The correlation coefficient was found to be 0.9996. The results were shown in Table 1 and Figure 2.

B. DRUG – POLYMER INTERACTION STUDIES

1) FTIR study

The IR Spectral analysis of benazepril hydrochloride alone showed that the principle peak were observed at wave number of 3504.43, 3111.28, 2978.18, 2805.01, 1735.99, 1676.20,1643.41, 1317.43, 1200.20, 858.35, and 765.77 (cm^{-1}) which indicates the presence of Carboxylic acid O-H stretch, Secondary amine N-H stretch, Methyl C-H stretch, Methylene C-H stretch, Carboxylic acid C=O stretch, Keto C=O stretch, Aromatic C=C ring stretch, RCOOR C-O stretch, R_2NH C-N stretch, Tetra sub. aromatic C-H stretch, and Mono sub

aromatic C-H stretch respectively. The results were shown in Figures3A, 3B, 3C, 3D, 3E, 3F & 3G. In the IR spectra of the physical mixture of benazepril hydrochloride with ES100, EL100 & EC the principle peak were observed nearly at the same wave number as in benazepril hydrochloride pure drug. However some additional peaks were observed with physical mixture, which could be due to the presence of polymers. The result of FTIR study suggested that there is no interaction between the drug and polymers used in the present study (Madhusudan Rao Y *et al*., 2007).

2) Differential Scanning Calorimetry Study

DSC study had been widely used as a rapid thermal method for examining the drug excipients compatibility because this method is fast, versatile and uses only mg of sample. When comparing the thermal behaviors of the pure drug, and the physical mixture of drug in polymer, analysis of the DSC curves can predict any interactions. In DSC thermogram the endothermic melting transition of benazepril hydrochloride was observed at 162.27 ºC. The results of DSC study were shown in Figure 4A, 4B, 4C, 4D, 4E, 4F, 4G & 4H. No shifts in the endothermic peak of Benazepril Hydrochloride or additional peaks were observed in the DSC thermogram of the physical mixture of Benazepril Hydrochloride and polymers (EC, ES100 & EL100) indicating that no chemical interaction between Benazepril Hydrochloride and polymer occurred (Liang Fang *et al*., 2007).

C. FORMULATION OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCHES

Matrix type transdermal patches of benazepril hydrochloride were prepared by using polymers ethyl cellulose (EC), Eudragit S100 (ES100) and Eudragit L100 (EL100) in three different concentrations, (EC -2.5%, 3.0%, and 3.5% ; ES100 - 4.0%, 4.5%, and 5.0% ; EL100 - 4.5%, 5.0%, and 5.5%) with and without the addition of permeation enhancer 30% Dimethyl sulfoxide (DMSO). The patches were prepared by solvent casting technique using Polyethylene glycol 400 (PEG 400 5% for ES100, 2.5% for EL100) and Dibutyl phthalate (30% for EC) as a plasticizer (Gajanan Darwhekar *et al*., 2011). The compositions were shown in Table 2.

D. EVALUATION OF TRANSDERMAL PATCHES

1) Physical Appearance

The use of mercury substrate method for the preparation of transdermal patches yielded opaque, smooth, flexible, nonsticky and uniform patches in case of ethylcellulose polymer where as transparent, smooth, flexible, nonsticky and uniform patches in case of Eudragit polymer. The results were shown in Table 3. The results indicated that the method used for casting the film on a mercury substrate was found to be satisfactory.

2) Weight Variation

The weight of the patches were ranged from 13.60 ± 0.4898 mg to 21.80 ± 0.2160 mg for ethylcellulose patches prepared with DMSO (F_1, F_3, and F_5), 12.20 ± 0.4326 mg to 20.06 ± 0.7930 mg for ethylcellulose patches prepared without DMSO (F_2, F_4, and F_6), 24.76 ± 0.4189 mg to 30.80 ± 0.5099 mg for Eudragit S100 patches prepared with DMSO (F_7, F_9, and F_{11}), 23.46 ± 0.6182 mg to 28.60 ± 1.4445 mg for Eudragit S100 patches prepared without DMSO (F_8, F_{10}, and F_{12}), 11.70 ± 0.7118 mg to 26.03 ± 0.8178 mg for Eudragit L100 patches prepared with DMSO (F_{13}, F_{15}, and F_{17}), 11.30 ± 0.0471 mg to 24.90 ± 0.3741 mg for Eudragit L100 patches prepared without DMSO (F_{14}, F_{16}, and F_{18}). The results were shown in Table 4 and Figure 5.

From the results it was observed that the weight of the patches containing dimethylsulfoxide (F_1, F_3, F_5, F_7, F_9, F_{11}, F_{13}, F_{15}, and F_{17}) was greater than that of patches prepared without permeation enhancer (F_2, F_4, F_6, F_8, F_{10}, F_{12}, F_{14}, F_{16}, and F_{18}) (Rupesh V. Chikhale *et al*., 2011).

3) Thickness of the patch

The thickness of patches varied from 0.07 ± 0.005 mm to 0.31 ± 0.017 mm. The results were shown in Table 4 and in Figure 6. The thickness of the patches were found to increased in the following order,

F_1 - EC 2.5% with DMSO (0.08 ± 0.005 mm) < F_3 - EC 3.0% with DMSO (0.09 ± 0.005 mm) < F5 - EC 3.5% with DMSO (0.10 ± 0.011 mm)

F_2 - EC 2.5% without DMSO (0.07 ± 0.005 mm) < F_4 - EC 3.0% without DMSO (0.08 ± 0.005 mm) < F_6 - EC 3.5% without DMSO (0.09 ± 0.010 mm)

F_7 - ES100 4.0% with DMSO (0.26 ± 0.005 mm) < F_9 - ES100 4.5% with DMSO (0.27 ± 0.015 mm) < F_{11} - ES100 5.0% with DMSO (0.29 ± 0.005 mm)

F_8 - ES100 4.0% without DMSO (0.20 ± 0.010 mm) < F10 - ES100 4.5% without DMSO (0.23 ± 0.005 mm) < F_{12} - ES100 5% without DMSO (0.28 ± 0.011 mm)

F_{13} - EL100 4.5% with DMSO (0.13 ± 0.011 mm) < F15 - EL100 5.0% with DMSO (0.15 ± 0.005 mm) < F_{17} - EL100 5.5% with DMSO (0.18 ± 0.005 mm)

F_{14} - EL100 4.5% without DMSO (0.12 ± 0.015 mm) < F16 - EL100 5.0% without DMSO (0.14 ± 0.010 mm) < F_{18} - EL100 5.5% without DMSO (0.17 ± 0.017 mm)

From the results it was observed that thickness of the patches increased with increase in concentration of polymer. The minimum standard deviation values assumed that the process used for preparing the patches was capable of giving reproducible results (Gajanan darwhekar *et al*., 2011).

4) Folding Endurance

Folding endurance measures the ability of patch to withstand rupture. The results of folding endurance were shown in Table 4. The folding endurance of Eudragit patches (F_7 to F_{18}) was ranged from 32.00 ± 0.6432 to 247.0 ± 1.6329 where as it was ranged from 04.33 ± 0.4714 to 35.30 ± 1.2472 for the patches prepared with Ethylcellulose (F_1 to F_6). From the results it was observed that the folding endurance was found to be high in patches containing Eudragit polymer when compared to the patches containing ethyl cellulose polymer (Meenakshi Bharkatiya *et al.,* 2010). This was due to less film forming property of cellulose derivative when compared to Eudragit (Hemangi J. Patel *et al*., 2009)

5) Flatness

All the formulations showed 100% flatness. The results of flatness study showed that none of the formulation had the difference in the strip lengths before and after longitudinal cut and thus they could maintain a smooth surface when applied on to the skin (Gilhotra Ritu Mehra *et al*., 2011).

6) Percentage Moisture Content

The percentage moisture content in the patches was found to be low and ranged from 1.52 ± 0.061 to 3.66 ± 0.43%. The results were shown in Table 4 and Figure 7.

The moisture content in the patches were found to be in the following order,

F_1 -EC 2.5% with DMSO (2.95 ± 0.086 %) > F_3 -EC 3.0% with DMSO (1.86 ± 0.123 %) > F_5 -EC 3.5% with DMSO (1.69 ± 0.173 %)

F_2 -EC 2.5% without DMSO (2.50 ± 0.061 %) > F_4 -EC 3.0% without DMSO (2.15 ± 0.171 %) > F_6 - EC 3.5% without DMSO (1.52 ± 0.061 %)

F_7 -ES100 4.0% with DMSO (3.66 ± 0.430 %) > F_9 -ES100 4.5% with DMSO (3.42 ± 0.291%) > F11 - ES100 5.0% with DMSO (2.23 ± 0.232 %)

F_8 -ES100 4.0% without DMSO (3.56 ± 0.318 %) > F10 -ES100 4.5% without DMSO (2.39 ± 0.158 %) > F_{12} -ES100 5% without DMSO (1.99 ± 0.323 %)

F_{13} -EL100 4.5% with DMSO (3.03 ± 0.174 %) > F_{15} -EL100 5.0% with DMSO (2.52 ± 0.196 %) > F_{17} -EL100 5.5% with DMSO (2.34 ± 0.070 %)

F_{14} -EL100 4.5% without DMSO (2.66 ± 0.039 %) > F16 -EL100 5.0% without DMSO (2.48 ± 0.399 %) > F_{18} -EL100 5.5% without DMSO (2.25 ± 0.037 %)

From the results it was observed that the moisture content decreases with increase in concentration of hydrophobic polymers in all the formulations. This could be due to increased hydrophobic nature of polymeric matrix which has less affinity for water which is resulted in decreased moisture content (Kevin C Garala *et al.,* 2009). The moisture content in all the formulations helps them to remain stable and protect from being a completely dried and brittle film (Madhusudan Rao Y *et al*., 2007).

7) Estimation of drug content

The drug content of all the patches (F_1 to F_{18}) was in the range of 75.78 ± 0.63 to 95.95 ± 1.09%. The results were shown in Table 4. The results suggest that the process employed to prepare the patches shown uniform drug content, with minimum batch variability (Gajanan Darwhekar *et al*., 2011).

8) *Invitro* drug release studies

The *invitro* drug release studies were performed using the modified paddle over disc apparatus (USP apparatus 5). The objective was to estimate, characterize and rationalize the drug release from matrix films (Gilhotra Ritu Mehra *et al*., 2011). The results of invitro drug release studies were shown in Tables 5, 6, 7, 8, 9, 10 and Figures 8A, 8B, 8C, 8D, 8E, 8F, 8G, 8H, 8I, 8J, 8K, 8L, 8M, 8N & 8O.

The cumulative percentage drug release of formulations containing Ethylcellulose (2.5%, 3.0%, and 3.5%) with DMSO in 12 hours were found to be 75.51% (F_1), 60.55% (F_3), and 43.85% (F_5).

The cumulative percentage drug release of formulations containing Ethylcellulose (2.5%, 3.0%, and 3.5%) without DMSO in 12 hours were found to be 52.47% (F_2), 44.68% (F_4), and 37.15% (F_6).

The cumulative percentage drug release of formulations containing Eudragit S100 (4.0%, 4.5%, and 5.0%) with DMSO in 12 hours were found to be 81.26% (F_7), 67.20% (F_9), and 63.48% (F_{11}).

The cumulative percentage drug release of formulations containing Eudragit S100 (4.0%, 4.5%, and 5.0%) without DMSO in 12 hours were found to be 71.97% (F_8), 60.86% (F_{10}), and 49.76% (F_{12}).

The cumulative percentage drug release of formulations containing Eudragit L100 (4.5%, 5.0%, and 5.5%) with DMSO in 12 hours were found to be 76.37% (F_{13}), 64.75% (F_{15}), and 58.93% (F_{17}).

The cumulative percentage drug release of formulations containing Eudragit L100 (4.5%, 5.0%, and 5.5%) without DMSO in 12 hours were found to be 65.83% (F_{14}), 59.78% (F_{16}), and 53.33% (F_{18}).

a) Effect of polymer on invitro drug release

The cumulative percentage drug release of the formulations containing Eudragit L100 (F_{13} - 76.37%, F_{14} - 65.83%, F_{15} - 64.75%, F_{16} - 59.78%, F_{17} - 58.93%, and F_{18} - 53.33%) and Eudragit S100 (F_7 - 81.26%, F_8 -71.97%, F_9 - 67.20%, F_{10} - 60.86%, F_{11} - 63.48%, and F_{12} - 49.76%) showed higher drug release when compared to the formulations containing Ethylcellulose (F_1 - 75.51%, F_2 - 52.47%, F_3 - 60.55%, F_4 - 44.68%, F_5 - 43.85, and F_6 - 37.15%). This was due to larger cavity size in Eudragit polymeric network and thus a faster mode of diffusion of drug from the Eudragit containing formulations as compared to the Ethylcellulose containing formulations (Biswajit Mukherjee *et al.,* 2005).

b) Effect of polymer concentration on invitro drug release

The cumulative percentage drug release of all the formulations decreased in the following order,

F_1 - EC 2.5% with DMSO (75.51%) > F_3 - EC 3.0% with DMSO (60.55%) > F_5 - EC 3.5% with DMSO (43.85%)

F_2 - EC 2.5% without DMSO (52.47%) > F_4 - EC 3.0% without DMSO (44.68) > F_6 - EC 3.5% without DMSO (37.15%)

F_7 - ES100 4.0% with DMSO (81.26%) > F_9 - ES100 4.5% with DMSO (67.2%) > F_{11} - ES100 5.0% with DMSO (63.48%)

F_8 - ES100 4% without DMSO (71.97%) > F_{10} - ES100 4.5% without DMSO (60.86%) > F_{12} - ES100 5% without DMSO (49.76%)

F_{13} - EL100 4.5% with DMSO (76.37%) > F_{15} - EL100 5% with DMSO (64.75%) > F_{17} - EL100 5.5% with DMSO (58.93%)

F_{14} - EL100 4.5% without DMSO (65.83%) > F_{16} - EL100 5% without DMSO (59.78%) > F_{18} - EL100 5.5% without DMSO (53.33%)

The cumulative percentage drug release of the formulations containing EC – 2.5%, 3.0%, and 3.5% (F_1 to F_6), ES100 – 4.0%, 4.5%, and 5.0% (F_7 to F_{12}) and EL100 – 4.0%, 5.0%,

and 5.5% (F_{13} to F_{18}) were found to decrease with increase in concentration of EC, EL100, and ES100 in the formulations respectively (Kevin C Garala *et al*., 2009). This could be due to increased hydrophobic nature of polymeric matrix which has less affinity for water this resulted in decreased in thermodynamic activity of drug in the film and thus decreased drug release (Gattani S.G. *et al.,* 2007).

c) Effect of plasticizer on invitro drug release

The cumulative percentage drug release from Eudragit S100 formulations containing 5.0% PEG 400 (F_7 - 81.26%, F_8 -71.97%, F_9 - 67.20%, F_{10} - 60.86%, F_{11} - 63.48%, and F_{12} - 49.76%) were found to be greater than that of Eudragit L100 formulations containing 2.5% of Polyethylene glycol 400 (F_{13} - 76.37%, F_{14} - 65.83%, F_{15} - 64.75%, F_{16} - 59.78%, F_{17} - 58.93%, and F_{18} - 53.33%). This could be due to the addition of plasticizer change the physicochemical properties of the patches such as their polymer tortuosity and porosity which can influence drug diffusion (Fusun Acarturk *et al.,* 1996).

d) Effect of permeation Enhancer on invitro drug release

The cumulative percentage drug release from the formulations containing permeation enhancer (F_1 - 75.51%, F_3 - 60.55%, F_5 - 43.85%, F_7 - 81.26%, F_9 - 67.20%, F_{11} - 63.48%, F_{13} - 76.37%, F_{15} - 64.75%, and F_{17} - 58.93%) was found to greater than that of the formulations prepared without permeation enhancer (F_2 - 52.47%, F_4 - 44.68%, F_6 - 37.15%, F_8 -71.97%,

F_{10} - 60.86%, F_{12} - 49.76%, F_{14} - 65.83%, F_{16} - 59.78%, and F_{18} - 53.33%). This could be due to miscibility and the solution properties of the permeation enhancer which were responsible for the enhanced drug release (Hemangi J. Patel *et al*., 2009).

9) Study of drug release kinetics

The description of dissolution profile by a model function has been attempted using different kinetics (zero order, first order, Higuchi square root model, Korsmeyer's Peppas model and Hixson Crowell model) (M. Guyot *et al.,* 2000). All the formulations ($F_1 – F_{18}$) showed zero order release kinetics (Fusan Acarturk *et al*., 1996). The correlation coefficient values (R^2) were found to be in the range of 0.980 - 0.999. The results were shown in Table 12 and Figures 9A, 9B, 9C, 9D, 9E, 9F, 9G, 9H, 9I, 9J, 9K, 9L, 9M, 9N & 9O. All the formulations were followed Higuchi mechanism. The correlation coefficient (R^2) values were found to be in the range of 0.977 – 0.998 (Hemangi J, Patel *et al*., 2009, P. Minghetti *et al*., 2000). The n values ($0.5 < n < 1$) of Korsmeyer's peppas model indicated that the release of drug from all the patches followed anomalous transport (Ashu Mittal *et al*., 2009).

10) *Ex vivo* permeability study

The formulation F_{17} (EL100 5.5% with DMSO) was selected for *ex vivo* permeability study on the basis of *invitro* release kinetics. For the determination of effect of permeation enhancer and polymer on *ex vivo* permeability formulation F_{18} (EL100 5.5% without DMSO) and

pure drug solution were selected for the *ex vivo* permeability study. The results of *ex vivo* drug permeation study were shown in Tables 11, 13 and 14, Figures 10A, 10B, 10C & 10D.

The cumulative amount permeated from the formulation F_{17} (EL100 5.5% with DMSO) was found to be 0.9312 mg in 12 hours where as it was found to be 0.8079 mg for F_{18} (EL100 5.5% without DMSO) and 1.1750 mg for pure drug solution.

The flux achieved during *ex vivo* permeation study were found to be 0.09228 mg/cm^2/hr, 0.05852 mg/cm^2/hr and 0.05373 mg/cm^2/hr for pure drug solution, formulation F_{17} (EL100 5.5% with DMSO) and F_{18} (EL100 5.5% without DMSO) respectively.

The lag time was found to be 1.0 hour for formulation F_{17} (EL100 5.5% with DMSO) where as it was found to be 1.1 hour for formulation F_{18} (EL100 5.5% without DMSO) and 0.9 hour for pure drug solution.

The permeability coefficient was found to be 0.0169 h^{-1} for formulation F_{17} (EL100 5.5% with DMSO), 0.0111 h^{-1} for formulation F_{18} (EL100 5.5% without DMSO) and 0.0239 h^{-1} for pure drug solution.

The result of drug permeation from transdermal patches of benazepril hydrochloride through the rat abdominal skin confirmed that benazepril hydrochloride was released from the

formulation and permeated through the skin and hence could possibly permeate through human skin (Madhusudan Rao Y *et al*., 2007).

a) Effect of permeation enhancer on ex vivo permeability study

The formulation F_{17} exhibited the greatest cumulative amount of drug permeation (0.9312 mg) which was significantly high ($P < 0.01$) when compared to lowest value (0.8079 mg) observed with the formulation F_{18} in 12 hours.

F_{17} - EL100 5.5% with DMSO (0.9312 mg) > F_{18} - EL100 5.5% without DMSO (0.8079 mg)

The flux of formulation F_{17} (EL100 5.5% with DMSO) was found to be greater than that of formulation F_{18} (EL100 5.5% without DMSO). The flux were found to be the following order,

F_{17} EL100 5.5% with DMSO (0.05852 mg/cm^2/hr) > F_{18} EL100 5.5% without DMSO (0.05373 mg/cm^2/hr)

The lag time of formulation F_{17} (EL100 5.5% with DMSO) was found to be less than that of formulation F_{18} (EL100 5.5% without DMSO). The lag time were found to be the following order,

F_{17} EL100 5.5% with DMSO (1.0 hour) < F_{18} EL100 5.5% without DMSO (1.1hour)

The above results indicated that the presence of dimethylsulfoxide in the formulation F17 (EL100 5.5% with DMSO) increase the permeation of drug when compared to the formulation

F_{18} (EL100 5.5% without DMSO). Because dimethylsulfoxide being a powerful solvent can mix isothermally with water, it can displace water from the lipid head groups, creating gaps around these head group, it also capable of displacing water bound to protein head group. More over due to its solvent power high level of sulfoxide within the membrane can improve drug partitioning and thus increase the permeation. (Chandra Amrish *et al*., 2009).

b) Effect of polymer on ex vivo permeability study

The pure drug solution exhibited the greatest cumulative amount of drug permeation and flux which were significantly high ($P < 0.001$) when compared to the lowest value observed with the formulations (F_{17} and F_{18}).

The cumulative amount of drug permeation were found to be the following order,

Pure drug solution (1.1750 mg) > F_{17} - EL100 5.5% with DMSO (0.9312 mg) > F_{18} - EL100 5.5% without DMSO (0.8079 mg)

The flux were found to be the following order,

Pure drug solution (0.09228 mg/cm^2/hr) > F_{17} EL100 5.5% with DMSO (0.05852 mg/cm^2/hr) > F_{18} EL100 5.5% without DMSO (0.05373 mg/cm^2/hr)

The decline in permeation observed with the film when compared to the pure drug solution could be due to drug depletion in the region of the film in contact with the skin. The

results obtained with the ex vivo permeability study suggest that the element of control of drug permeation across skin was the release of the drug from the formulation (Patrizia santi *et. al*., 2007).

c) Release kinetics of ex vivo permeability

The description of permeation profile by a model function has been attempted using different kinetics (zero order, first order, Higuchi square root model, Korsmeyer's peppas model and Hixson Crowell model) (M. Guyot et al., 2000). The results were shown in Table 15 and Figures 11A, 11B, 11C, 11D & 11E.

Formulations F_{17} and F_{18} showed zero order release kinetics (Fusan Acarturk *et al.,* 1996). The correlation coefficient values (R^2) were found to be 0.975 and 0.990. The formulations F_{17} and F_{18} were followed Higuchi mechanism. The correlation coefficient (R^2) values were found to be 0.955 and 0.979. The n values of Korsmeyer's peppas model indicated that the release of drug from the formulations F_{17} (n = 0.524) followed Fickian diffusion mechanism (Jatin Kumar Pruthi *et al*., 2011) where as formulation F_{18} (n = 0.604) followed anomalous transport (Ashu Mittal *et al*., 2009).

d) Dose design

Theoretical drug input required was calculated using the following mathematical equation, (Ashu Mittal *et al*., 2009)

Drug input (theoretical) = $C_{ss} \times K_e \times V_d$

= 138 µg/hr

The flux achieved after ex vivo permeability study of 1cm^2 of patch was found to be **0.05852 mg/cm^2/hr.** Hence by increasing the surface area of the formulation to **2.36 cm^2** the required rate of flux may be achieved.

11) Histopathological study

The results of histopathological studies were shown in Table 16 and Figures 12A, 12B, 12C, 12D & 12E.

The sample A (normal healthy rat abdominal skin) showed intact stratum corneum with muscle bundle and fat. The result indicated that there is no histological abnormalities could found in the skin section of the control group (A).

The sample B (rat abdominal skin in contact with phosphate buffered saline pH 7.4 for 12 hours) showed fibro fatty tissue, inflammatory cell infiltrate and oedema. Identical changes were presented in the samples from group C (rat abdominal skin in contact with benazepril

hydrochloride pure drug solution for 12 hours), group D (rat abdominal skin in contact with F_{17} formulation containing EL100 5.5% with DMSO), group E (skin in contact with F_{18} formulation containing EL100 5.5% without DMSO). The results indicated that there is no anatomical degradation which was observed in the morphology of skin sample exposed to formulation ($F_{17,}$ F_{18}) and pure drug solution. (Gasem K.A.M. *et al.,* 2010). Hence the enhanced permeation of formulation containing dimethylsulfoxide could be due to its solvent action which resulted in increased drug partition in to the skin (pooja Mathur *et al*., 2011).

E. SCANNING ELECTRON MICROSCOPY

The surface morphology of the transdermal patch before and after invitro drug release study was scanned using a scanning electron microscope. The results were shown in Figures 13A & 13B.

Figure 13A showed surface morphology of the patch before *ex vivo* permeation study which indicated that the uniform distribution of drug in the polymer matrix.

Figure 13B showed surface morphology of the patch after *ex vivo* permeation study. From the result it was observed that the film maintained the elastic nature after the release of drug molecule without affecting the other parts of the patch (Priyanka Arora *et al*., 2002).

CHAPTER XI

SUMMARY AND CONCLUSION

In the present work an attempt has been made to formulate and evaluate the transdermal patches of benazepril hydrochloride using various types of polymers (Eudragit L100, Eudragit S100 and ethylcellulose).

The results of compatibility studies by Fourier transform infrared spectroscopy and differential scanning Calorimetry showed no interaction between the drug and polymers.

The polymers Eudragit L100 and Eudragit S100 used for the formulation of transdermal patches showed good film forming property when compared to ethylcellulose.

The patches prepared by using Eudragit L100 and Eudragit S100 were thin, flexible, smooth and transparent where as the patches prepared by using ethylcellulose were thin, flexible, smooth and opaque.

The weight variation test showed less variation in weight and suggesting uniform distribution of drug and polymer over the mercury surface.

The thickness of the transdermal patches increased with increasing the concentration of polymers Eudragit L100, Eudragit S100 and ethylcellulose.

Eudragit patches showed good flexibility and folding endurance properties when compared to ethylcellulose patches.

All the formulations showed 100% flatness which indicating that all patches could maintain a smooth surface when applied on to the skin.

The moisture content in the patches were found to low and the formulations containing high concentrations of hydrophobic polymer showed low percentage of moisture content.

The drug content analysis showed minimum variations suggesting uniform distribution of drug.

In vitro release studies suggested that drug release of all the formulations decreased with increase in polymer concentration.

The kinetic analysis of *in vitro* drug release studies suggested that all the formulations followed zero order release kinetics and non Fickian diffusion mechanism.

The results of *ex vivo* permeation studies showed that the addition of dimethyl sulfoxide increased the permeation of drug and the required target flux can be achieved by increasing surface area of the patch to 2.36 cm^2.

The results of histopathological study suggested that the addition of permeation enhancer increased the permeation of drug through the skin by means of its solvent action and thus it did not cause anatomical degradation in the skin.

Surface morphological studies by scanning electron microscopy showed that the patch showed uniform smooth surface and did not lose integrity after release.

Hence it was concluded that solvent casting method using mercury substrate was useful for successful development of matrix type transdermal patches of benazepril hydrochloride. The controlled release of drug from the transdermal patches suggested that the frequency of administration can be reduced. The transdermal patches can improve the bioavailability of the benazepril hydrochloride by avoiding hepatic first pass metabolism. Further *in vivo* investigation was required to correlate *ex vivo* permeation studies for the development of suitable transdermal system of benazepril hydrochloride. The present investigation suggested that the matrix type transdermal patches of benazepril hydrochloride could be explored for the management of hypertension.

Table 1

CALIBRATION CURVE OF BENAZEPRIL HYDROCHLORIDE USING PHOSPHATE BUFFERED SALINE PH 7.4

Sl.no	Concentration µg/ml	Absorbance ± S.D*
1	2	0.043 ± 0.0000
2	4	0.083 ± 0.0016
3	6	0.128 ± 0.0049
4	8	0.163 ±0.0016
5	10	0.198 ± 0.0008
6	12	0.247 ± 0.0058
7	14	0.285 ± 0.0049
8	16	0.333 ± 0.0060
9	18	0.371 ± 0.0071
10	20	0.414 ± 0.0104
		γ = 0.9996

***n = 3**

Table 2

COMPOSITION OF TRANSDERMAL PATCHES OF BENAZEPRIL HYDROCHLORIDE

Sl.no	Formulation Code	Polymer	Weight (mg)	Dibutyl phthalate (% w/w of polymer)	Poly ethylene glycol (% w/w of polymers)	Dimethyl sulfoxide (% w/w of polymer)
1	F_1	EC	125	30	_	30
2	F_2	EC	125	30	_	_
3	F_3	EC	150	30	_	30
4	F_4	EC	150	30	_	_
5	F_5	EC	175	30	_	30
6	F6	EC	175	30	_	_
7	F_7	ES100	200	_	5	30
8	F_8	ES100	200	_	5	_
9	F_9	ES100	225	_	5	30
10	F_{10}	ES100	225	_	5	_
11	F_{11}	ES100	250	_	5	30
12	F_{12}	ES100	250	_	5	_
13	F_{13}	EL100	225	_	2.5	30
14	F_{14}	EL100	225	_	2.5	_
15	F_{15}	EL100	250	_	2.5	30
16	F_{16}	EL100	250	_	2.5	_
17	F_{17}	EL100	275	_	2.5	30
18	F_{18}	EL100	275	_	2.5	_

Table 3

S.NO	FORMULATION CODE	PHYSICAL APPEARANCE
1	F_1-EC 2.5% (with DMSO)	opaque, smooth, nonsticky and flexible
2	F_2- EC 2.5% (without DMSO)	opaque, smooth, nonsticky and flexible
3	F_3- EC 3.0% (with DMSO)	opaque, smooth, nonsticky and flexible
4	F_4- EC 3.0% (without DMSO)	opaque, smooth, nonsticky and flexible
5	F_5- EC 3.5% (with DMSO)	opaque, smooth, nonsticky and flexible
6	F_6- EC 3.5% (without DMSO)	opaque, smooth, nonsticky and flexible
7	F_7- ES100 4.0% (with DMSO)	Transparent, smooth, nonsticky and flexible
8	F_8- ES100 4.0% (without DMSO)	Transparent, smooth, nonsticky and flexible
9	F_9- ES100 4.5% (with DMSO)	Transparent, smooth, nonsticky and flexible
10	F_{10}- ES100 4.5%(without DMSO)	Transparent, smooth, nonsticky and flexible
11	F_{11}- ES100 5.0% (with DMSO)	Transparent, smooth, nonsticky and flexible
12	F_{12}- ES100 5.0%(without DMSO)	Transparent, smooth, nonsticky and flexible
13	F_{13}- EL100 4.5% (with DMSO)	Transparent, smooth, nonsticky and flexible
14	F_{14}- EL100 4.5%(without DMSO)	Transparent, smooth, nonsticky and flexible
15	F_{15}- EL100 5.0% (with DMSO)	Transparent, smooth, nonsticky and flexible
16	F_{16}- EL100 5.0%(without DMSO)	Transparent, smooth, nonsticky and flexible
17	F_{17}- EL100 5.5% (with DMSO)	Transparent, smooth, nonsticky and flexible
18	F_{18}- EL100 5.5%(without DMSO)	Transparent, smooth, nonsticky and flexible

Table 4 CHARACTERISATION OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCHES

Formulation Code	Weight ± S.D* (mg)	Thickness ± S.D* (mm)	Folding Endurance ± S.D* (No. of times)	Moisture content ± S.D* (%)	Drug content ± S.D* (%)
F_1	13.60 ± 0.4898	0.08 ± 0.005	09.33 ± 0.4714	2.95 ± 0.086	84.00 ± 2.52
F_2	12.20 ± 0.4326	0.07 ± 0.005	04.33 ± 0.4714	2.50 ± 0.061	85.83 ± 1.01
F_3	18.06 ± 1.2656	0.09 ± 0.005	06.66 ± 0.4714	1.86 ± 0.123	89.38 ± 2.29
F_4	15.70 ± 0.5099	0.08 ± 0.005	05.66 ± 0.4714	2.15 ± 0.171	84.67 ± 2.89
F_5	21.80 ± 0.2160	0.10 ± 0.011	35.30 ± 1.2472	1.69 ± 0.173	75.78 ± 0.63
F_6	20.06 ± 0.7930	0.09 ± 0.010	07.33 ± 0.4714	1.52 ± 0.061	87.36 ± 1.24
F_7	24.76 ± 0.4189	0.26 ± 0.005	46.00 ± 0.8164	3.66 ± 0.430	92.08 ± 0.86
F_8	23.46 ± 0.6182	0.20 ± 0.010	32.00 ± 0.6432	3.56 ± 0.318	81.98 ± 1.86
F_9	29.40 ± 0.6539	0.27 ± 0.015	189.3 ± 1.2472	3.42 ± 0.291	91.40 ± 1.24
F_{10}	26.23 ± 0.3299	0.23 ± 0.005	145.0 ± 3.9665	2.39 ± 0.158	84.50 ± 1.86
F_{11}	30.80 ± 0.5099	0.29 ± 0.005	152.6 ± 2.0548	2.23 ± 0.232	82.48 ± 3.33
F_{12}	28.60 ± 1.4445	0.28 ± 0.011	149.5 ± 2.3342	1.99 ± 0.323	87.36 ± 0.71
F_{13}	11.70 ± 0.7118	0.13 ± 0.011	247.0 ± 1.6329	3.03 ± 0.174	83.32 ± 2.29
F_{14}	11.30 ± 0.0471	0.12 ± 0.015	197.0 ± 0.8164	2.66 ± 0.039	87.32 ± 0.63
F_{15}	25.53 ± 1.0498	0.15 ± 0.005	221.0 ± 1.6329	2.52 ± 0.196	95.95 ± 1.09
F_{16}	22.13 ± 2.6599	0.14 ± 0.010	171.7 ± 0.9428	2.48 ± 0.399	85.68 ± 1.26
F_{17}	26.03 ± 0.8178	0.18 ± 0.005	129.3 ± 0.4714	2.34 ± 0.070	77.77 ± 1.24
F_{18}	24.90 ± 0.3741	0.17 ± 0.017	105.7 ± 1.2472	2.25 ± 0.037	86.52 ± 1.04

*n = 3

Table 5

IN VITRO RELEASE PROFILE OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH

Time (Hours)	Cumulative % Drug Release		
	Formulation Code		
	F_1 EC 2.5% (with DMSO)	F_2 EC 2.5% (without DMSO)	F_3 EC 3.0% (with DMSO)
	Mean ± SD*(%)	Mean ± SD*(%)	Mean ± SD* (%)
0.25	13.92 ± 2.47	10.08 ±2.22	10.42 ±2.09
0.50	15.92 ±2.13	12.29 ±2.71	13.04 ±1.20
0.75	18.52 ±2.42	13.04 ±2.97	14.29 ±1.69
1.00	20.43 ±2.09	13.85 ±3.03	15.93 ±0.77
1.50	22.49 ±1.47	17.92 ±3.02	17.70 ±1.39
2.00	25.61 ±1.90	19.57 ±1.56	19.11 ±0.55
2.50	28.51 ±3.18	22.80 ±1.56	20.53 ±0.21
3.00	32.50 ±1.11	24.48 ±1.90	22.21 ±0.55
3.50	35.32 ±1.09	26.18 ±1.25	24.13 ±0.60
4.00	41.02 ±2.87	28.48 ±1.79	25.82 ±0.32
4.50	44.49 ±2.24	29.83 ±2.16	28.36 ±1.01
5.00	46.16 ±1.98	30.35 ±1.67	31.22 ±1.58
5.50	47.97 ±1.14	31.79 ±2.56	32.99 ±2.09
6.00	49.91 ±1.02	34.40 ±2.00	34.49 ±2.21
6.50	52.82 ±2.60	35.19 ±2.55	35.99 ±2.67
7.00	54.41 ±1.89	38.61 ±3.08	38.46 ±2.59
7.50	58.07 ±2.52	39.9 ±2.66	40.46 ±1.52
8.00	60.89 ±2.54	41.07 ±2.81	41.92 ±1.62
8.50	63.25 ±2.49	42.36 ±2.42	44.42 ±1.25
9.00	65.86 ±2.19	43.42 ±2.72	46.47 ±1.28
9.50	68.00 ±2.21	46.05 ±1.25	47.42 ±1.25
10.0	69.69 ±1.06	47.49 ±1.69	51.26 ±1.27
10.5	71.73 ±1.20	48.81 ±2.20	53.60 ±1.50
11.0	73.19 ±0.86	50.26 ±1.35	55.53 ±1.47
11.5	74.52 ±1.19	51.61 ±1.73	58.46 ±2.24
12.0	75.51 ±1.08	52.47 ±1.21	60.55 ±1.01

*n = 3

Table 6

IN VITRO RELEASE PROFILE OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH

Time (Hours)	Cumulative % Drug Release		
	Formulation Code		
	F_4 EC 3.0% (without DMSO)	F_5 EC 3.5% (with DMSO)	F_6 EC 3.5% (without DMSO)
	Mean ± SD* (%)	Mean ± SD* (%)	Mean ± SD* (%)
0.25	07.80 ± 1.61	05.79 ±2.28	03.99 ± 1.52
0.50	09.62 ± 1.49	09.10±2.20	05.19 ± 2.00
0.75	11.02 ± 1.78	12.60 ± 3.27	06.41 ± 1.49
1.00	13.00 ± 1.41	14.99 ± 4.78	09.8 0± 1.48
1.50	14.75 ± 1.21	15.82 ± 2.74	11.29 ± 1.08
2.00	16.27 ± 0.87	17.48 ± 3.33	12.56 ± 1.19
2.50	17.56 ± 0.97	18.42 ± 2.61	13.35 ± 0.74
3.00	19.33 ± 0.37	17.51 ± 4.34	15.10 ± 1.50
3.50	20.99 ± 0.30	22.23 ± 2.46	16.99 ± 1.50
4.00	22.31 ± 0.47	23.43 ± 2.19	18.64 ± 1.55
4.50	23.63 ± 1.32	24.52 ± 2.94	19.58 ± 1.44
5.00	24.84 ± 1.17	27.05 ± 1.93	20.52 ± 1.61
5.50	26.05 ± 1.74	28.18 ± 1.57	21.83 ± 2.06
6.00	28.23 ± 1.45	30.16 ± 2.76	23.03 ± 2.30
6.50	30.30 ± 1.45	30.92 ± 3.75	24.35 ± 2.75
7.00	31.66 ± 2.23	32.92 ± 3.33	25.20 ± 1.88
7.50	33.51 ± 2.69	34.14 ± 3.43	27.25 ± 2.23
8.00	35.12 ± 3.20	34.92 ± 3.70	28.6 0± 2.12
8.50	36.15 ± 3.18	35.34 ± 4.71	29.83± 1.82
9.00	37.78 ± 2.55	37.93 ± 5.33	31.16 ± 1.86
9.50	39.06 ± 2.64	38.72 ± 4.95	33.03 ± 1.66
10.0	39.98 ± 2.44	39.64 ± 5.47	33.56 ± 1.44
10.5	41.03 ± 2.44	40.92 ± 6.07	33.50 ± 1.26
11.0	42.68 ± 2.15	41.86 ± 6.11	34.51 ± 1.62
11.5	43.86 ± 1.63	43.03 ± 6.30	36.13 ± 1.98
12.0	44.68 ± 2.03	43.85 ± 6.14	37.15 ± 2.13

*n = 3

Table 7

IN VITRO RELEASE PROFILE OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH

Time (Hours)	Cumulative % Drug Release		
	Formulation Code		
	F_7 ES100 4.0% (with DMSO)	F_8 ES100 4.0% (without DMSO)	F_9 ES100 4.5% (with DMSO)
	Mean ± SD*(%)	Mean ± SD*(%)	Mean ± SD*(%)
0.25	17.52±1.89	10.32 ± 0.85	08.52 ± 2.24
0.50	25.17±2.66	16.13 ± 0.34	10.24 ± 2.40
0.75	26.99 ± 2.21	18.75 ± 1.52	11.38 ± 1.91
1.00	28.71 ± 2.92	20.05 ± 0.52	13.49 ± 2.07
1.50	31.62 ± 4.59	21.96 ± 1.56	15.48 ± 2.29
2.00	32.88 ± 4.62	24.48 ± 0.35	17.73 ± 3.23
2.50	34.98 ± 3.81	25.93 ± 0.76	19.38 ± 3.14
3.00	36.97 ± 4.02	27.87 ± 0.76	21.49 ± 2.25
3.50	40.29 ± 2.50	29.22 ± 1.20	24.28 ± 2.90
4.00	42.30 ± 2.49	31.18 ± 1.78	27.65 ± 2.62
4.50	45.17 ± 3.15	33.39 ± 1.82	30.08 ± 1.68
5.00	48.29 ± 4.34	35.85 ± 1.55	32.89 ± 2.39
5.50	50.11 ± 5.10	38.56 ± 0.74	35.71 ± 3.37
6.00	51.21 ± 5.11	40.80 ± 1.56	37.57 ± 2.82
6.50	54.12 ± 5.37	41.98 ± 1.12	39.47 ± 3.57
7.00	58.00 ± 7.72	44.48 ± 0.92	42.67 ± 3.39
7.50	60.60 ± 7.28	47.84 ± 1.50	46.49 ± 4.43
8.00	62.83 ± 7.26	51.09 ± 0.80	48.06 ± 4.82
8.50	64.84 ± 7.85	53.28 ± 0.90	52.63 ± 3.64
9.00	67.93 ± 7.20	58.12 ± 0.74	55.67 ± 1.77
9.50	69.73 ± 7.49	59.87 ± 1.75	58.25 ± 0.84
10.0	73.05 ± 6.03	62.22 ± 2.02	60.35 ± 1.74
10.5	75.39 ± 5.92	64.58 ± 3.83	63.79 ± 2.34
11.0	77.81 ± 5.28	66.00 ± 2.18	64.48 ± 2.84
11.5	79.89 ± 4.60	69.09 ± 0.97	66.85 ± 2.26
12.0	81.26 ± 3.58	71.97 ± 0.77	67.20 ± 3.36

*n = 3

Table 8

IN VITRO RELEASE PROFILE OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH

Time (Hours)	Cumulative % Drug Release		
	Formulation Code		
	F_{10} ES100 4.5% (without DMSO)	F_{11} ES100 5.0% (with DMSO)	F_{12} ES100 5.0% (without DMSO)
	Mean ± SD* (%)	Mean ± SD* (%)	Mean ± SD* (%)
0.25	07.92 ± 1.47	06.24 ± 2.25	05.52 ± 1.03
0.50	10.00 ± 1.51	08.20 ± 2.97	07.25 ± 1.05
0.75	11.74 ± 1.03	10.04 ± 1.80	10.63 ± 1.37
1.00	14.08 ± 1.18	11.54 ± 1.92	12.13 ± 0.69
1.50	15.12 ± 2.38	12.44 ± 3.32	13.16 ± 1.49
2.00	18.08 ± 3.41	14.19 ± 3.37	14.79 ± 1.21
2.50	19.66 ± 3.24	15.18 ± 3.84	17.27 ± 1.58
3.00	20.45 ± 3.36	17.34 ± 5.05	19.64 ± 1.92
3.50	22.84 ± 3.90	19.12 ± 6.19	21.67 ± 2.61
4.00	24.40 ± 3.81	21.62 ± 6.62	03.31 ± 2.98
4.50	26.09 ± 3.50	23.78 ± 5.59	24.67 ± 2.89
5.00	29.12 ± 3.66	24.51 ± 6.56	26.01 ± 2.58
5.50	32.03 ± 4.86	26.08 ± 6.82	28.18 ± 2.88
6.00	34.60 ± 5.69	28.02 ± 6.86	29.05 ± 2.66
6.50	38.03 ± 5.96	30.33 ± 5.76	30.77 ± 2.36
7.00	39.67 ± 6.27	32.53 ± 6.13	33.09 ± 3.28
7.50	41.32 ± 4.51	34.98 ± 5.31	34.59 ± 3.34
8.00	45.26 ± 5.07	37.09 ± 4.00	36.81 ± 3.19
8.50	46.46 ± 5.05	40.64 ± 3.47	39.17 ± 3.01
9.00	48.74 ± 5.43	43.86 ± 4.09	40.21 ± 3.19
9.50	50.44 ± 5.31	47.33 ± 3.06	41.74 ± 3.24
10.0	52.17 ± 4.78	49.50 ± 2.53	43.40 ± 3.57
10.5	54.46 ± 3.33	51.32 ± 2.73	45.19 ± 3.29
11.0	56.54 ± 3.31	56.03 ± 1.66	46.94 ± 3.44
11.5	59.35 ± 0.78	60.28 ± 1.22	48.78 ± 1.79
12.0	60.86 ± 1.36	63.48 ± 2.28	49.76 ± 1.83

*n = 3

Table 9

IN VITRO RELEASE PROFILE OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH

Time (Hours)	Cumulative % Drug Release		
	Formulation Code		
	F_{13} EL100 4.5% (with DMSO)	F_{14} EL100 4.5% (without DMSO)	F_{15} EL100 5.0% (with DMSO)
	Mean ± SD*(%)	Mean ± SD*(%)	Mean ± SD*(%)
0.25	09.36 ± 1.56	07.20 ± 1.06	07.56 ± 1.06
0.50	12.05 ± 2.74	09.04 ± 1.07	09.40 ± 0.52
0.75	13.92 ± 3.25	10.53 ± 1.48	11.25 ± 1.07
1.00	16.16 ± 3.54	11.55 ± 1.34	12.39 ± 0.60
1.50	17.80 ± 3.36	13.24 ± 2.30	13.78 ± 0.86
2.00	19.70 ± 3.25	15.40 ± 2.55	16.62 ± 0.36
2.50	21.73 ± 3.67	17.29 ± 2.17	19.23 ± 0.33
3.00	23.65 ± 3.01	19.66 ± 2.27	21.85 ± 0.84
3.50	26.17 ± 3.30	22.41 ± 1.96	24.26 ± 1.18
4.00	27.64 ± 3.43	25.29 ± 2.65	27.02 ± 1.83
4.50	29.22 ± 3.23	27.74 ± 2.92	29.09 ± 2.25
5.00	30.82 ± 2.75	30.41 ± 3.37	31.29 ± 2.53
5.50	33.74 ± 2.74	32.37 ± 2.95	33.73 ± 3.27
6.00	35.84 ±1.91	33.99 ± 2.50	36.56 ± 3.96
6.50	38.80 ± 1.27	37.70 ± 3.31	39.15 ± 3.79
7.00	41.76 ± 1.80	40.49 ± 3.42	41.76 ± 4.20
7.50	45.47 ± 1.95	42.98 ± 3.72	43.42 ± 4.07
8.00	48.58 ± 2.79	45.02 ± 4.11	45.67 ± 5.02
8.50	50.76 ± 3.42	47.05 ± 4.13	47.97 ± 6.37
9.00	52.83 ± 2.64	48.90 ± 4.52	50.87 ± 6.64
9.50	55.03 ± 2.61	51.04 ± 4.53	52.57 ± 6.34
10.0	59.15 ± 2.96	54.19 ± 4.32	55.14 ± 5.08
10.5	61.74 ± 2.64	56.27 ± 3.54	57.82 ± 4.88
11.0	65.07 ± 3.05	59.80 ± 2.92	59.56 ± 3.38
11.5	69.12 ± 2.51	62.99 ± 2.56	62.26 ± 3.00
12.0	76.37 ± 2.10	65.83 ± 1.04	64.75 ± 0.93

*n = 3

Table 10

IN VITRO RELEASE PROFILE OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH

Time (Hours)	Cumulative % Drug Release		
	Formulation Code		
	F_{16} EL100 5.0% (without DMSO)	F_{17} EL100 5.5% (with DMSO)	F_{18} EL100 5.5% (without DMSO)
	Mean ± SD*(%)	Mean ± SD*(%)	Mean ± SD*(%)
0.25	06.12 ± 1.47	08.16 ± 0.34	08.4 ± 1.77
0.50	07.83 ± 1.37	10.00 ± 1.19	09.41 ± 1.94
0.75	10.16 ± 1.08	11.14 ± 0.85	11.14 ± 1.82
1.00	11.53 ± 1.34	13.24 ± 1.34	12.88 ± 1.80
1.50	13.28 ± 1.35	14.75 ± 1.21	14.63 ± 2.07
2.00	15.39 ± 1.58	16.88 ± 1.49	16.03 ± 2.24
2.50	17.17 ± 1.73	18.53 ± 1.54	18.16 ± 2.30
3.00	20.01 ± 1.42	20.07 ± 1.73	19.70 ± 2.26
3.50	21.55 ± 1.40	21.98 ± 1.79	21.60 ± 2.63
4.00	23.23 ± 1.75	23.77 ± 1.59	23.16 ± 2.80
4.50	25.55 ± 2.16	25.71 ± 1.59	24.72 ± 2.00
5.00	28.66 ± 4.03	28.18 ± 2.14	26.65 ± 2.30
5.50	31.09 ± 4.15	29.71 ± 1.59	28.72 ± 2.31
6.00	33.06 ± 4.17	32.27 ± 1.15	30.67 ± 2.76
6.50	36.12 ± 4.70	34.48 ± 0.95	31.80 ± 2.84
7.00	38.94 ± 4.57	36.70 ± 1.29	33.41 ± 3.18
7.50	40.90 ± 4.67	38.82 ± 1.87	35.02 ± 2.70
8.00	43.46 ± 5.25	41.15 ± 2.65	36.89 ± 3.23
8.50	45.43 ± 4.88	43.32 ± 3.05	38.64 ± 3.41
9.00	49.24 ± 5.47	45.23 ± 2.68	40.28 ± 3.72
9.50	51.01 ± 4.62	47.27 ± 2.70	41.69 ± 4.14
10.0	52.09 ± 3.43	49.31 ± 2.95	43.47 ± 3.64
10.5	54.16 ± 3.69	51.61 ± 2.68	45.62 ± 3.23
11.0	56.37 ± 3.52	54.29 ± 2.83	48.26 ± 2.80
11.5	59.23 ± 1.57	56.25 ± 2.10	50.66 ± 1.76
12.0	59.78 ± 1.45	58.93 ± 1.71	53.33 ± 1.33

*n = 3

Table 11

EXVIVO PERMEABILITY STUDY OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH AND PURE DRUG SOLUTION

Time (Hours)	Cumulative Amount of Drug Release (mg)		
	Formulation Code		
	F_{17} EL100 5.5% (with DMSO)	F_{18} EL100 5.5% (without DMSO)	Pure drug solution
	Mean ± SD*(%)	Mean ± SD*(%)	Mean ± SD*(%)
1	0.254 ± 0.026	0.182 ± 0.182	0.165 ± 0.022
2	0.292 ± 0.014	0.235 ± 0.025	0.209 ± 0.013
3	0.355 ± 0.011	0.308 ± 0.014	0.250 ± 0.016
4	0.463 ± 0.009	0.383 ± 0.037	0.311 ± 0.022
5	0.497 ± 0.004	0.446 ± 0.024	0.423 ± 0.023
6	0.527 ± 0.003	0.496 ± 0.010	0.492 ± 0.040
7	0.555 ± 0.007	0.528 ± 0.009	0.587 ± 0.024
8	0.610 ± 0.019	0.568 ± 0.005	0.692 ± 0.054
9	0.654 ± 0.006	0.611 ± 0.014	0.754 ± 0.019
10	0.764 ± 0.010	0.662 ± 0.021	0.892 ± 0.063
11	0.861 ± 0.015	0.732 ± 0.029	1.061 ± 0.058
12	0.931 ± 0.014	0.807 ± 0.020	1.175 ± 0.047

*n = 3

Table 12

KINETICS STUDY FOR DISOLUTION DATA OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCHES

Formulation code	Zero Order		First Order		Higuchi		Korsmeyer Peppas		Hixson Crowell		Release mechanism
	r^2	K^0 (h^{-1})	r^2	K_1 (h^{-1})	r^2	K_H ($h^{-1/2}$)	r^2	n	r^2	K_{HC} ($h^{-1/3}$)	
F1	0.986	5.378	0.985	-0.047	0.998	22.54	0.990	0.580	0.986	-0.131	NFD
F2	0.989	3.528	0.987	-0.023	0.996	14.78	0.993	0.530	0.987	-0.071	NFD
F3	0.997	4.053	0.977	-0.027	0.987	16.60	0.962	0.577	0.951	-0.085	NFD
F4	0.998	3.068	0.995	-0.018	0.997	12.78	0.980	0.537	0.979	-0.058	NFD
F5	0.996	2.967	0.990	-0.017	0.986	12.51	0.981	0.592	0.963	-0.057	NFD
F6	0.985	2.697	0.960	-0.015	0.991	11.32	0.988	0.575	0.984	-0.049	NFD
F7	0.994	5.043	0.969	-0.050	0.985	20.87	0.968	0.559	0.958	-0.134	NFD
F8	0.992	4.830	0.963	-0.038	0.977	19.79	0.948	0.554	0.947	-0.111	NFD
F9	0.997	5.225	0.978	-0.038	0.987	21.43	0.978	0.721	0.953	-0.115	NFD
F10	0.997	4.457	0.984	-0.030	0.991	18.32	0.967	0.654	0.958	-0.093	NFD
F11	0.980	4.509	0.936	-0.022	0.977	18.17	0.952	0.734	0.947	-0.092	NFD
F12	0.994	3.603	0.984	-0.038	0.987	14.99	0.978	0.616	0.953	-0.070	NFD
F13	0.991	5.032	0.945	-0.033	0.965	20.45	0.951	0.645	0.931	-0.112	NFD
F14	0.998	4.871	0.971	-0.033	0.992	19.96	0.987	0.769	0.954	-0.103	NFD
F15	0.998	4.806	0.984	-0.030	0.994	19.82	0.989	0.715	0.966	-0.102	NFD
F16	0.998	4.628	0.988	-0.030	0.993	19.04	0.981	0.727	0.961	-0.095	NFD
F17	0.999	4.181	0.982	-0.027	0.994	17.14	0.971	0.645	0.958	-0.085	NFD
F18	0.997	3.588	0.985	-0.022	0.992	14.79	0.977	0.595	0.963	-0.071	NFD

NFD – NON FICKIAN DIFFUSION

Table 13

EXVIVO PERMEATION PARAMETERS OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCHES AND PURE DRUG SOLUTION OBTAINED BY ANALYSES OF THE PERMEATION PROFILE

Formulation code	Accumulated amount at 12 Hour (mg/cm^2, Mean ± S.D*)	Flux ($mg/cm^2.h$)	Lag Time (Hour)	Permeability coefficient (h^{-1})
F17	0.9312 ± 0.0148	0.05852	1.0	0.0169
F18	0.8079 ± 0.0201	0.05373	1.1	0.0111
Pure drug solution	1.1750 ± 0.0478	0.09228	0.9	0.0239

*n = 3

Table 14

STATISTICAL ANALYSIS OF CUMULATIVE AMOUNT OF DRUG PERMEATED

Comparison	Difference	P value
F17 vs F18	0.1233	P < 0.01
F17 vs PD	-0.2439	P < 0.001
F18 vs PD	-0.3671	P < 0.001

95% Confidence Interval

Table 15

KINETICS STUDY FOR EXVIVO PERMEABILITY DATAOF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCHES AND PURE DRUG SOLUTION

Formulation code	Zero Order		First Order		Higuchi		Korsmeyer Peppas		Hixson Crowell		Release mechanism
	r^2	K^0 (mg/h^{-1})	r^2	K_1 (h^{-1})	r^2	K_H $(mg/h^{-1/2})$	r^2	n	r^2	K_{HC} $(h^{-1/3})$	
F17	0.975	1.170	0.970	-0.005	0.955	4.995	0.949	0.524	0.972	-0.019	FD
F18	0.990	1.074	0.991	-0.005	0.979	4.554	0.989	0.604	0.990	-0.017	NFD

NFD – NON FICKIAN DIFFUSION

Table 16

HISTOPATHOLOGICAL STUDY OF RAT ABDOMINAL SKIN

SAMPLE CODE	OBSERVATIONS
A(Untreated skin)	Skin with inter muscular oedema, inflammatory cell infiltrate
B(Skin treated with PBS P^{H}7.4)	Skin with fatty tissue and sparse inflammatory cell infiltrate
C(Skin treated with Benazepril Hydrochloride pure drug solution)	Skin with inflammatory cell infiltrate in the subcutaneous fat and oedema
D(Skin treated with transdermal patch containing EL100 5.5% with DMSO)	Skin with oedema with sparse inflammatory cell infiltrate
E(Skin treated with transdermal patch containing ES100 5.5% with DMSO)	Skin with oedema and dense inflammatory cell infiltrate

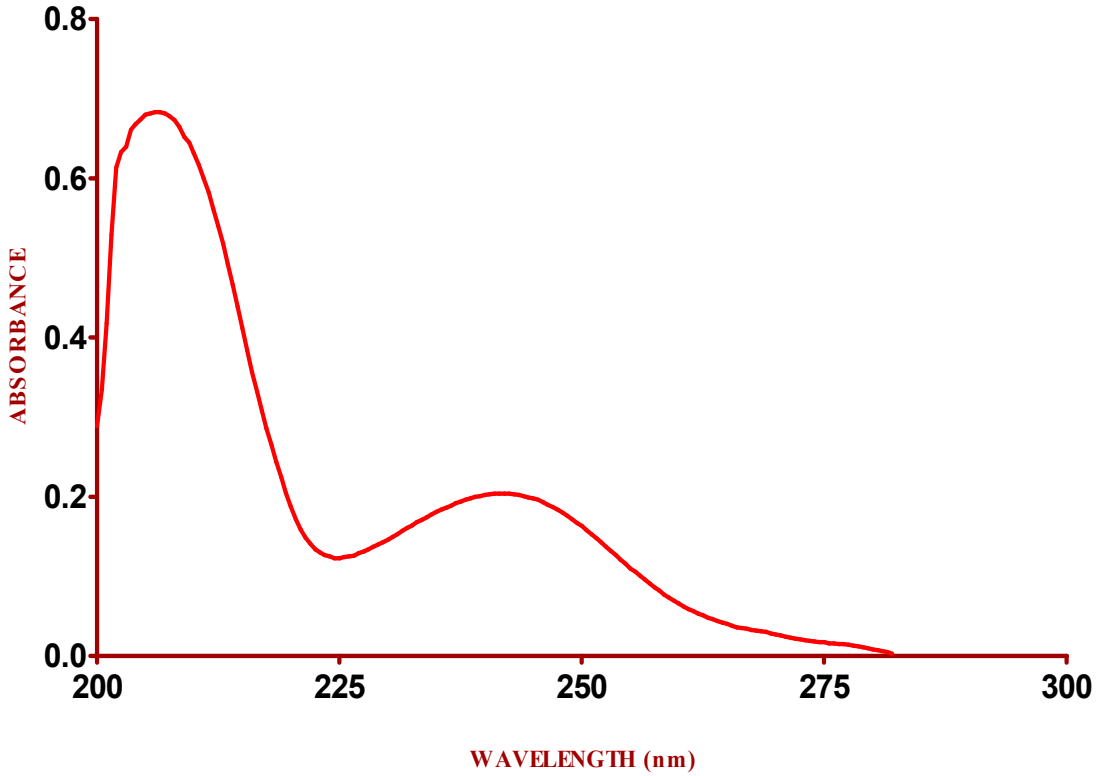


FIGURE 1 DETERMINATION OF λ_{max} OF BENAZEPRIL HYDROCHLORIDE IN PHOSPHATE BUFFERED SALINE OF pH 7.4

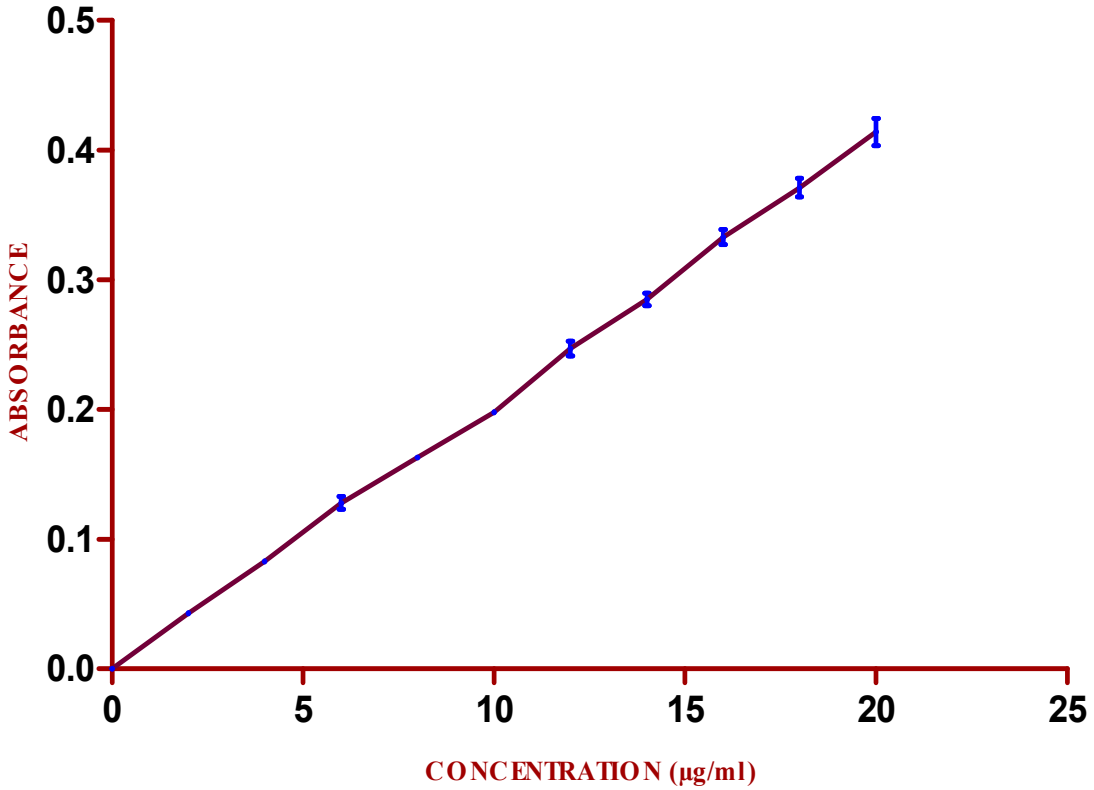
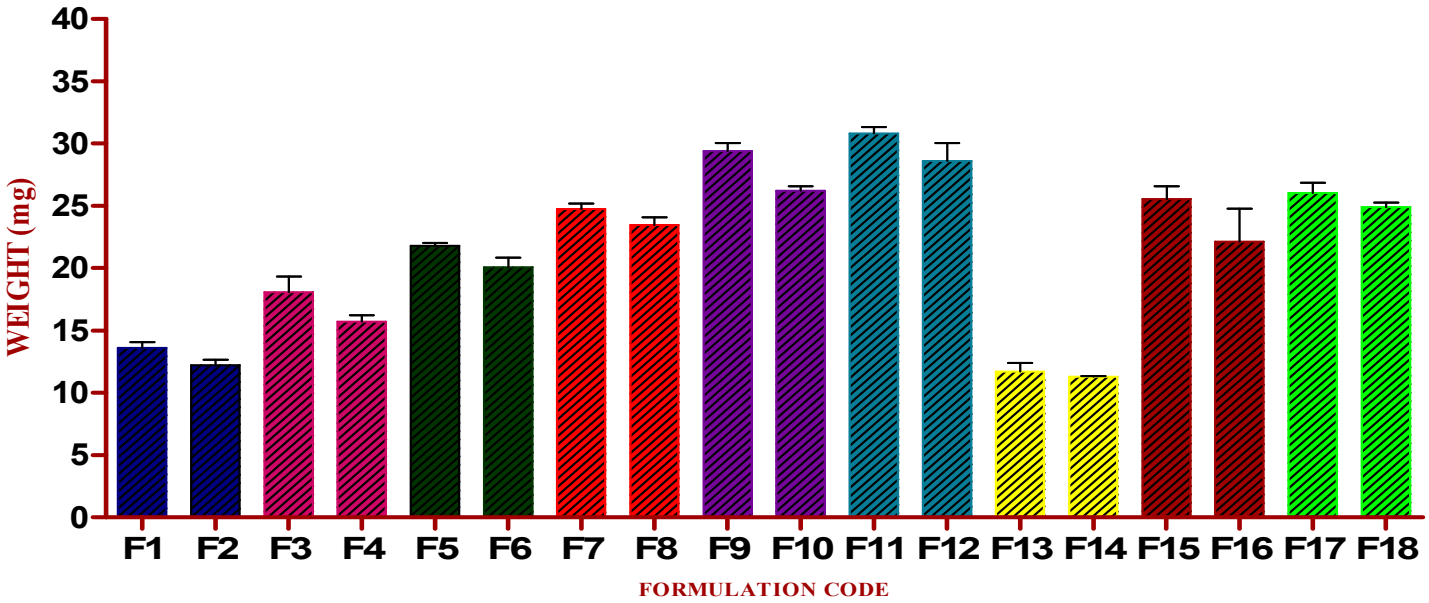


FIGURE 2 CALIBRATION CURVE FOR BENAZEPRIL HYDROCHLORIDE USING PHOSPHATE BUFFERED SALINE OF P^H - 7.4



F1-EC 2.5% (WITH DMSO)

F2-EC 2.5% (WITHOUT DMSO)

F3-EC 3.0% (WITH DMSO)

F4-EC 3.0% (WITHOUT DMSO)

F5-EC 3.5% (WITH DMSO)

F7-ES100 4.0% (WITH DMSO)

F8-ES100 4.0% (WITHOUT DMSO)

F9-ES100 4.5% (WITH DMSO)

F10-ES100 4.5% (WITHOUT DMSO)

F11-ES100 5.0% (WITH DMSO)

F13-EL100 4.5% (WITH DMSO)

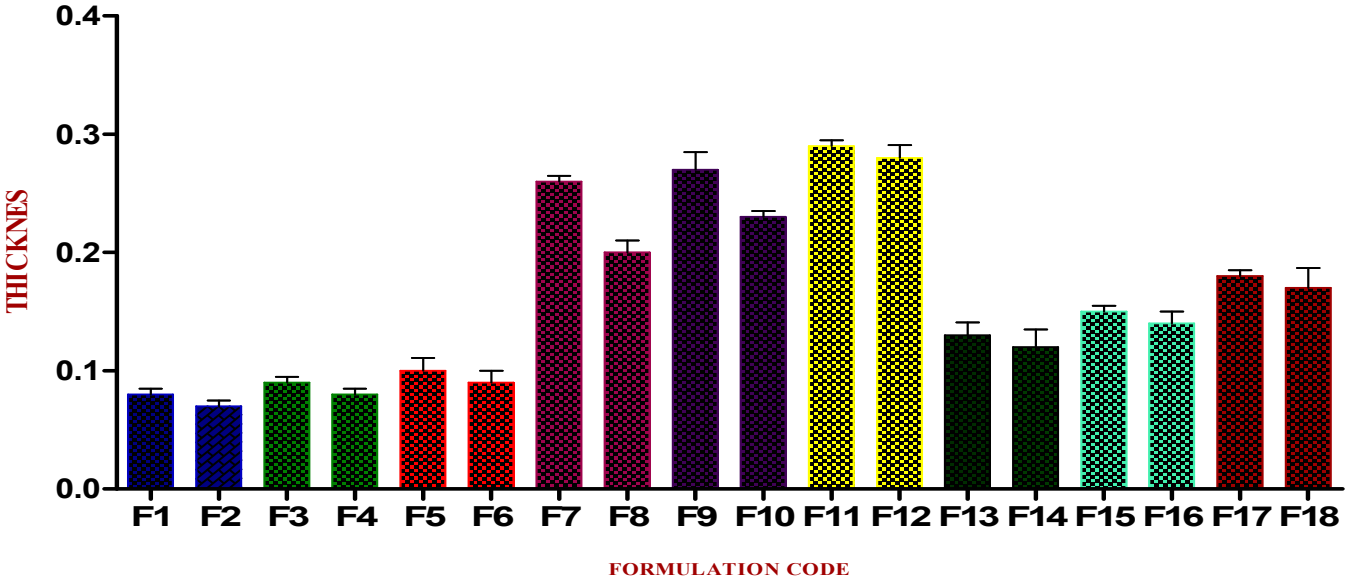
F14-EL100 4.5% (WITHOUT DMSO)

F15-EL100 5.0% (WITH DMSO)

F16-EL100 5.0% (WITHOUT DMSO)

F17-EL100 5.5% (WITH DMSO)

FIGURE 5 WEIGHT VARIATION OF ALL THE FORMULATIONS



F1-EC 2.5% (WITH DMSO)

F2-EC 2.5% (WITHOUT DMSO)

F3-EC 3.0% (WITH DMSO)

F4-EC 3.0% (WITHOUT DMSO)

F5-EC 3.5% (WITH DMSO)

F7-ES100 4.0% (WITH DMSO)

F8-ES100 4.0% (WITHOUT DMSO)

F9-ES100 4.5% (WITH DMSO)

F10-ES100 4.5% (WITHOUT DMSO)

F11-ES100 5.0% (WITH DMSO)

F13-EL100 4.5% (WITH DMSO)

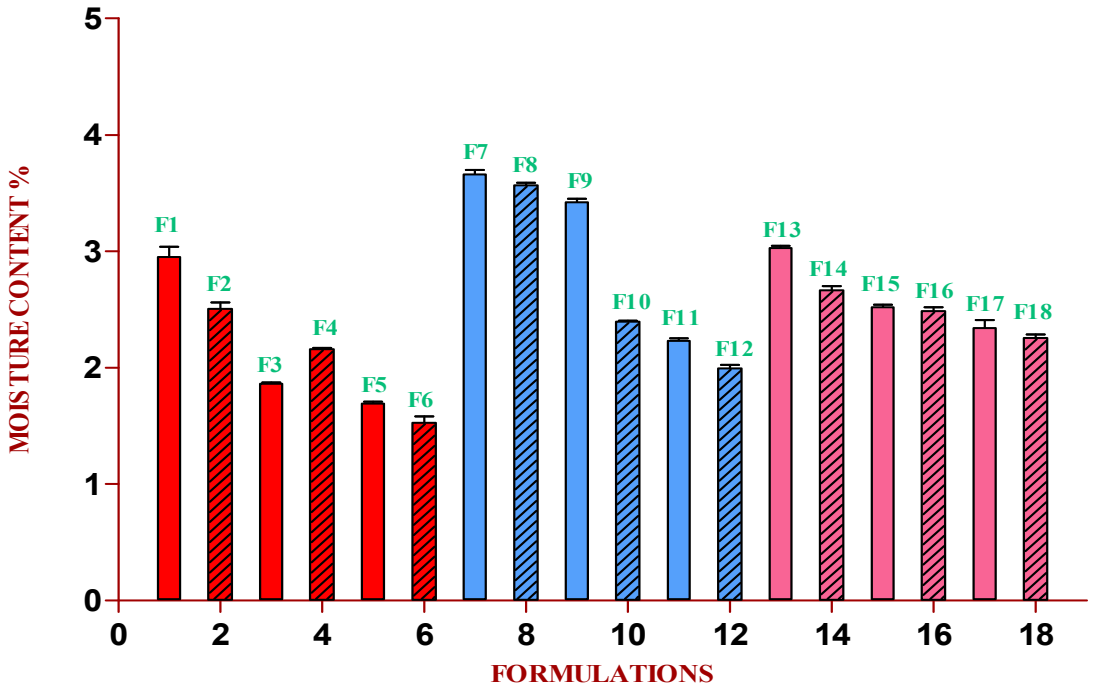
F14-EL100 4.5% (WITHOUT DMSO)

F15-EL100 5.0% (WITH DMSO)

F16-EL100 5.0% (WITHOUT DMSO)

F17-EL100 5.5% (WITH DMSO)

FIGURE 6 THICKNESS OF ALL THE FORMULATION



F1-EC 2.5% (WITH DMSO)

F2-EC 2.5% (WITHOUT DMSO)

F3-EC 3.0% (WITH DMSO)

F4-EC 3.0% (WITHOUT DMSO)

F5-EC 3.5% (WITH DMSO)

F6-EC 3.5% (WITHOUT DMSO)

F7-ES100 4.0% (WITH DMSO)

F8-ES100 4.0% (WITHOUT DMSO)

F9-ES100 4.5% (WITH DMSO)

F10-ES100 4.5% (WITHOUT DMSO)

F11-ES100 5.0% (WITH DMSO)

F12-ES100 5.0% (WITHOUT DMSO)

F13-EL100 4.5% (WITH DMSO)

F14-EL100 4.5% (WITHOUT DMSO)

F15-EL100 5.0% (WITH DMSO)

F16-EL100 5.0% (WITHOUT DMSO)

F17-EL100 5.5% (WITH DMSO)

F18-EL100 5.5% (WITHOUT DMSO)

FIGURE 7 MOISTURE CONTENT OF ALL FORMULATION

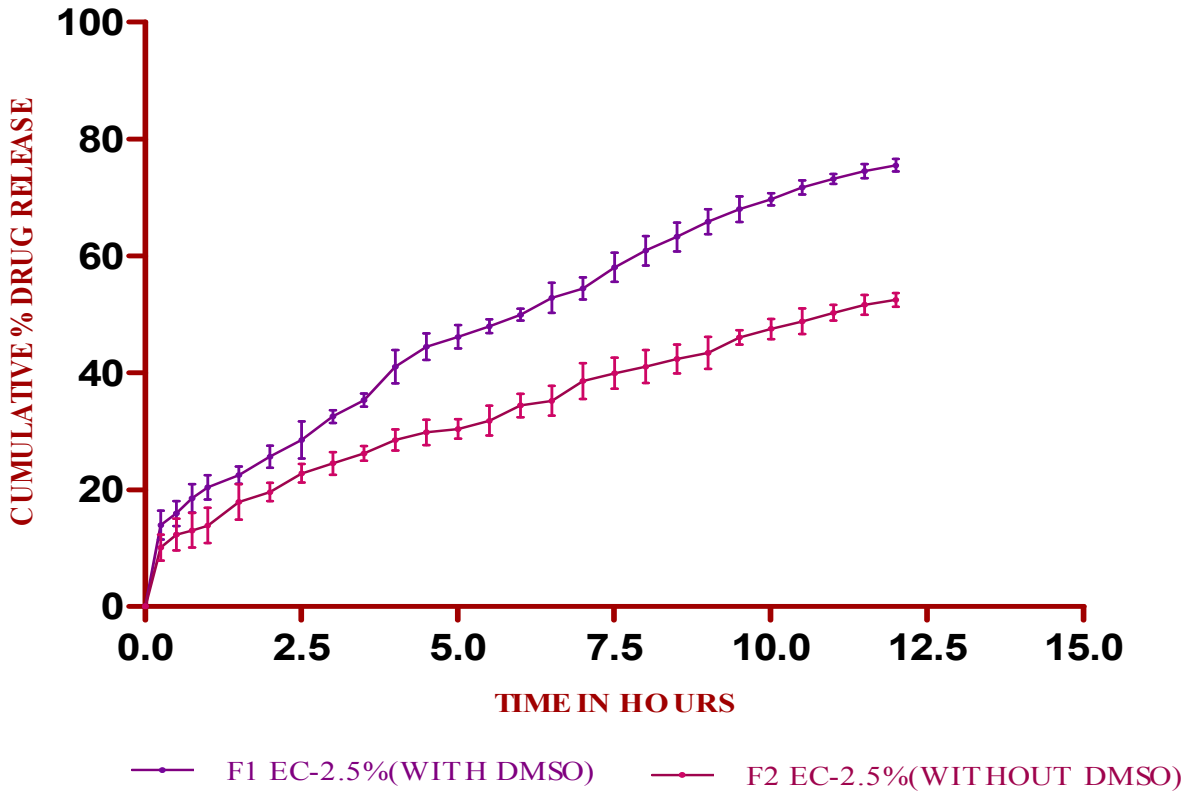


FIGURE 8A INVITRO RELEASE STUDY OF BENAZEPRIL HCL TRANSDERMAL PATCH CONTAINING ETHYLCELLULOSE -2.5% (WITH AND WITHOUT DMSO)

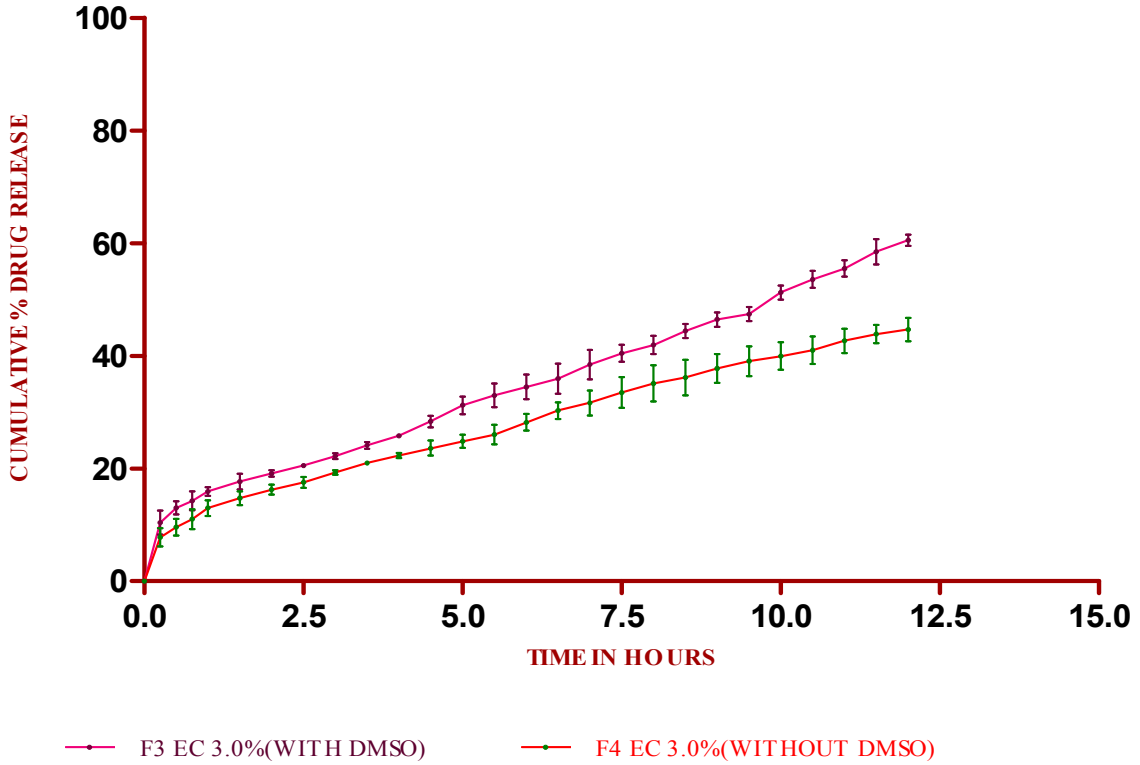


FIGURE 8B INVITRO RELEASE STUDY OF BENAZEPRIL HCL TRANSDERMAL PATCH CONTAINING ETHYLCELLULOSE -3.0 % (WITH AND WITHOUT DMSO)

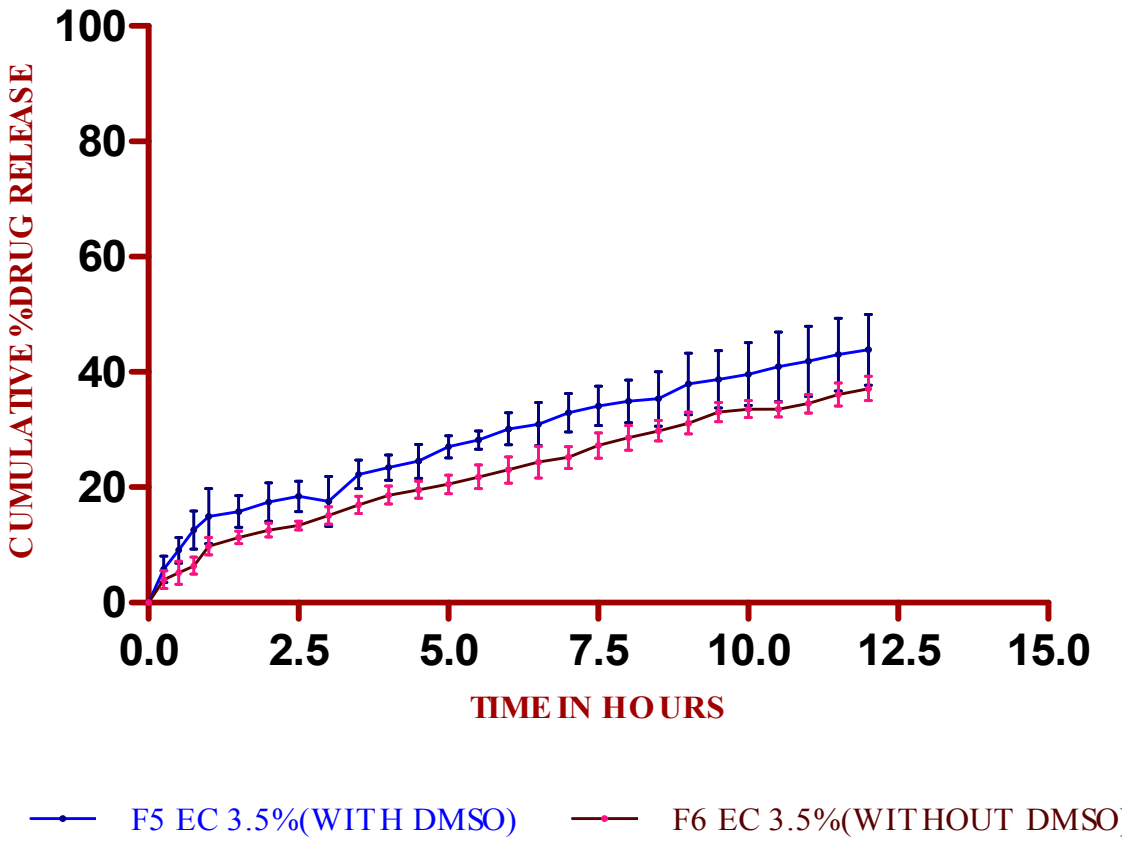


FIGURE 8C INVITRO RELEASE STUDY OF BENAZEPRIL HCL TRANSDERMAL PATCH CONTAINING ETHYLCELLULOSE -3.5 % (WITH AND WITHOUT DMSO)

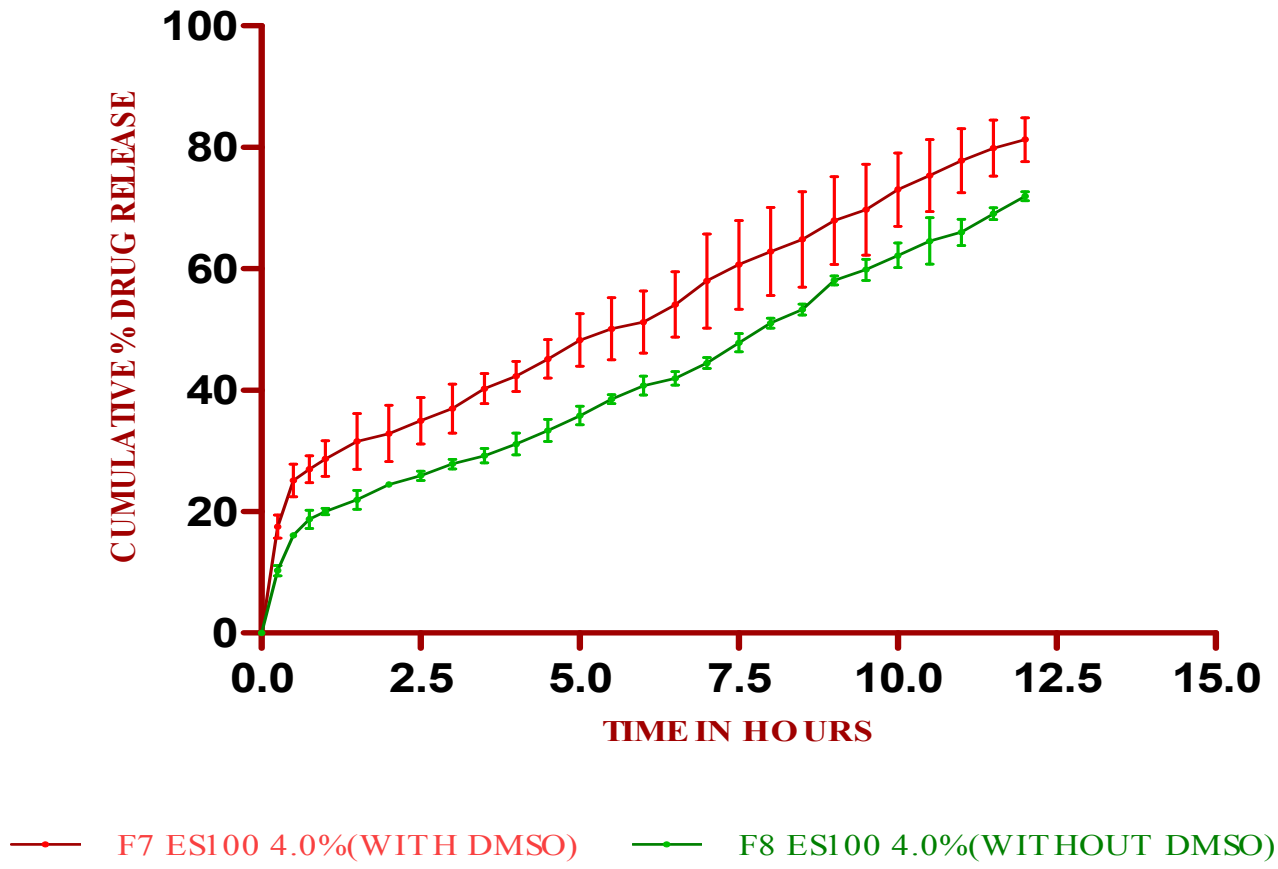


FIGURE 8D INVITRO RELEASE STUDY OF BENAZEPRIL HCL TRANSDERMAL PATCH CONTAINING EUDRAGIT S100 4.0% (WITH AND WITHOUT DMSO)

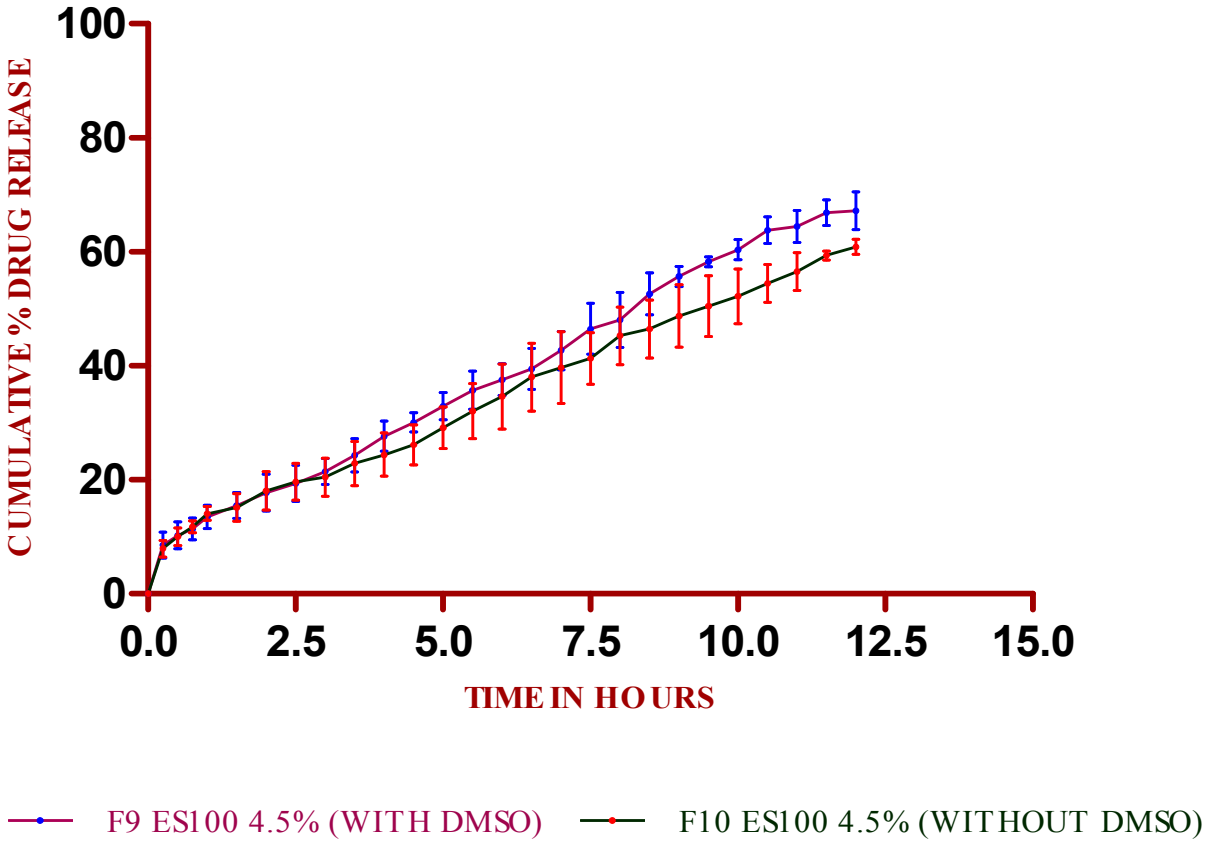


FIGURE 8E INVITRO RELEASE STUDY OF BENAZEPRIL HCL TRANSDERMAL PATCH CONTAINING EUDRAGIT S100 4.5% (WITH AND WITHOUT DMSO)

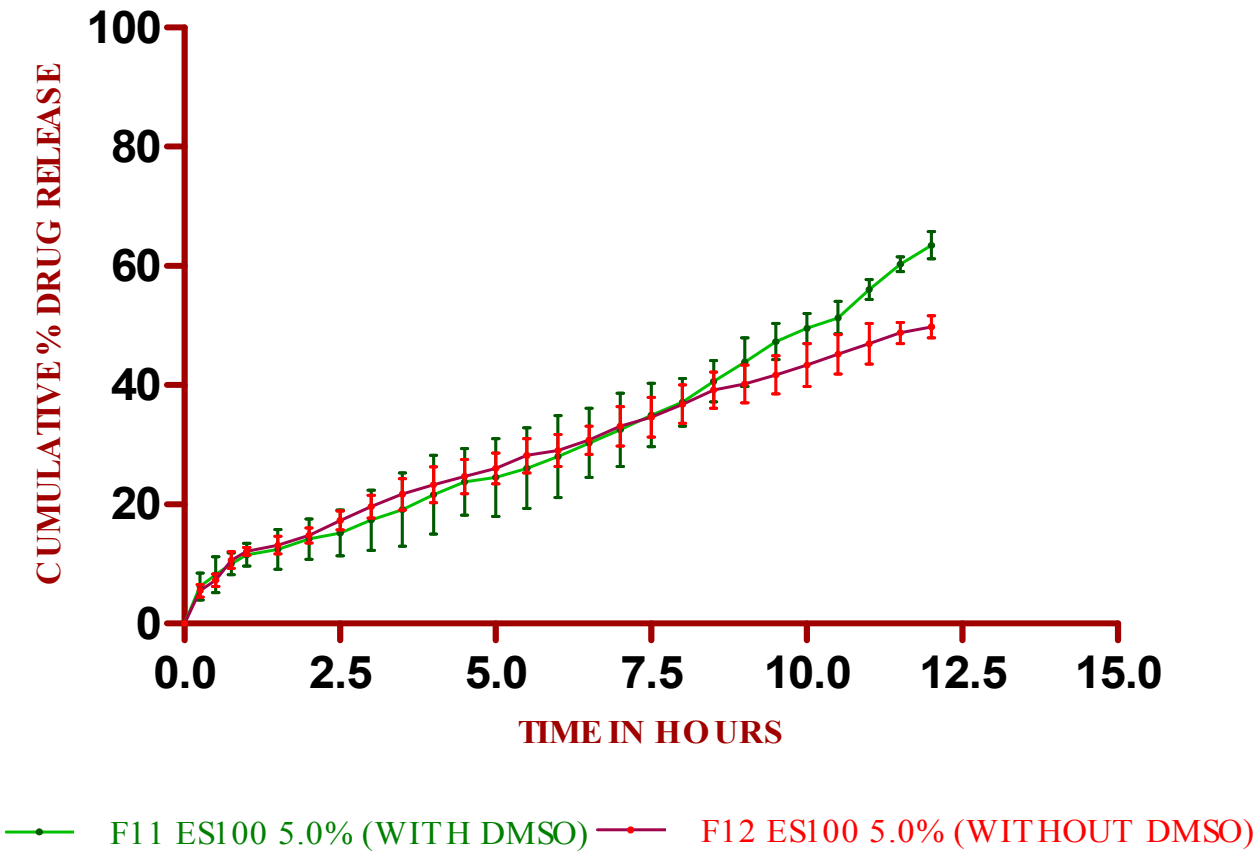


FIGURE 8F INVITRO RELEASE STUDY OF BENAZEPRIL HCL TRANSDERMAL PATCH CONTAINING EUDRAGIT S100 5.0 % (WITH AND WITHOUT DMSO)

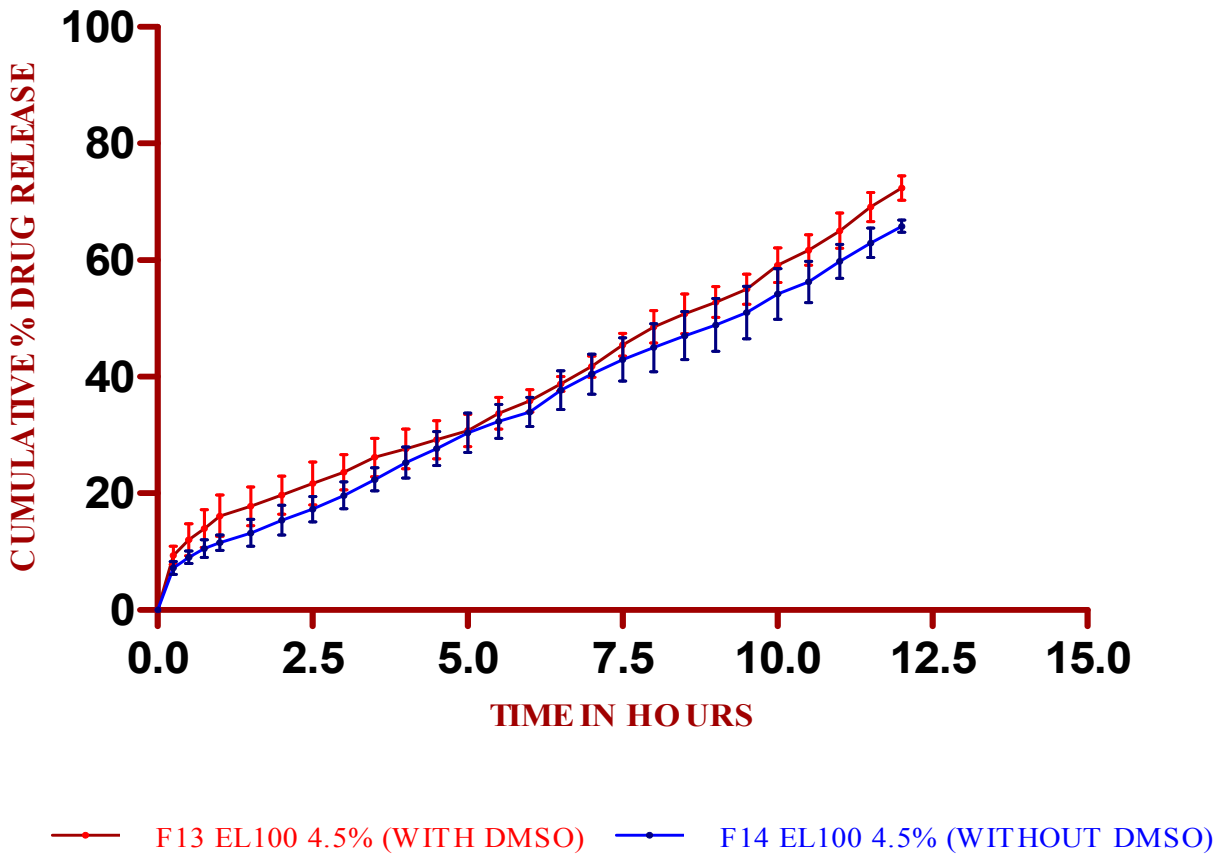


FIGURE 8G INVITRO RELEASE STUDY OF BENAZEPRIL HCL TRANSDERMAL PATCH CONTAINING EUDRAGIT L100 4.5% (WITH AND WITHOUT DMSO)

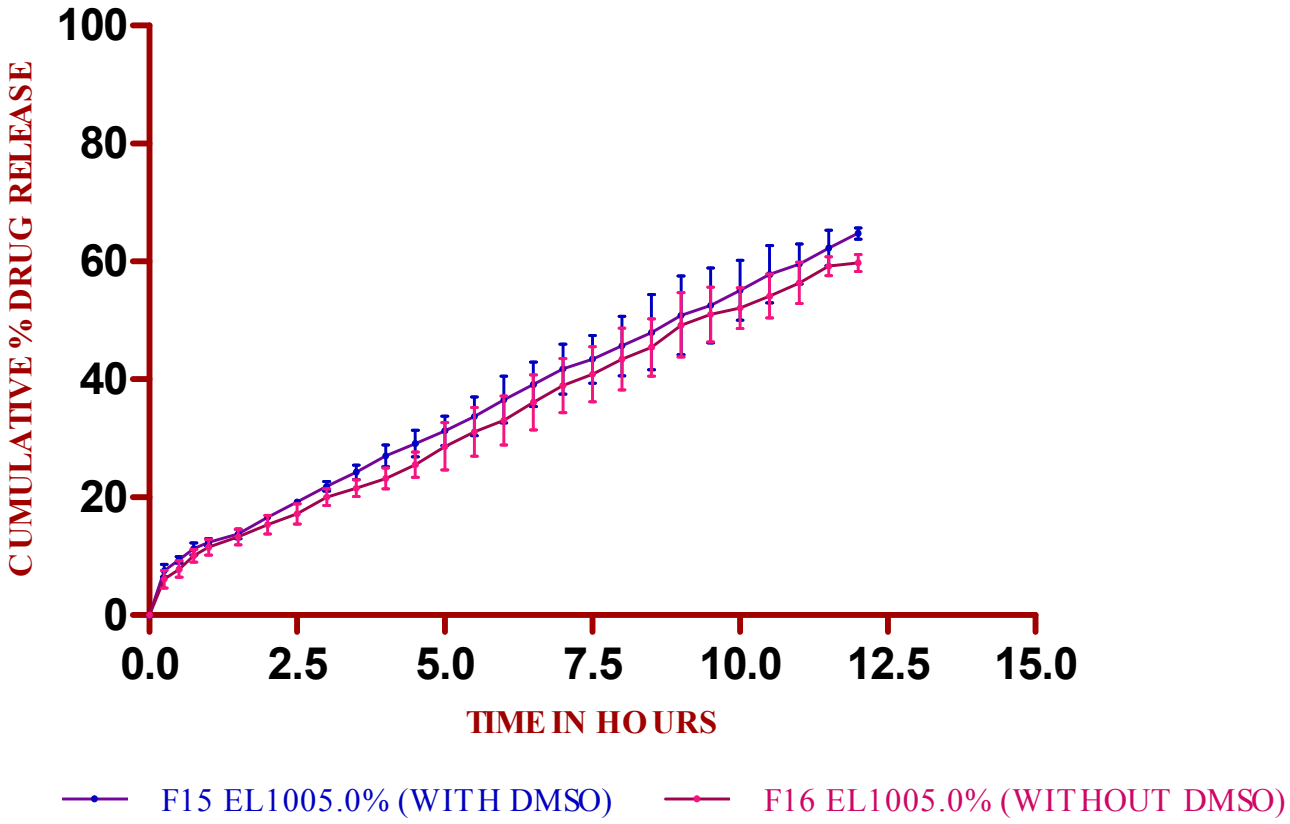


FIGURE 8H INVITRO RELEASE STUDY OF BENAZEPRIL HCL TRANSDERMAL PATCH CONTAINING EUDRAGIT L100 5.0% (WITH AND WITHOUT DMSO)

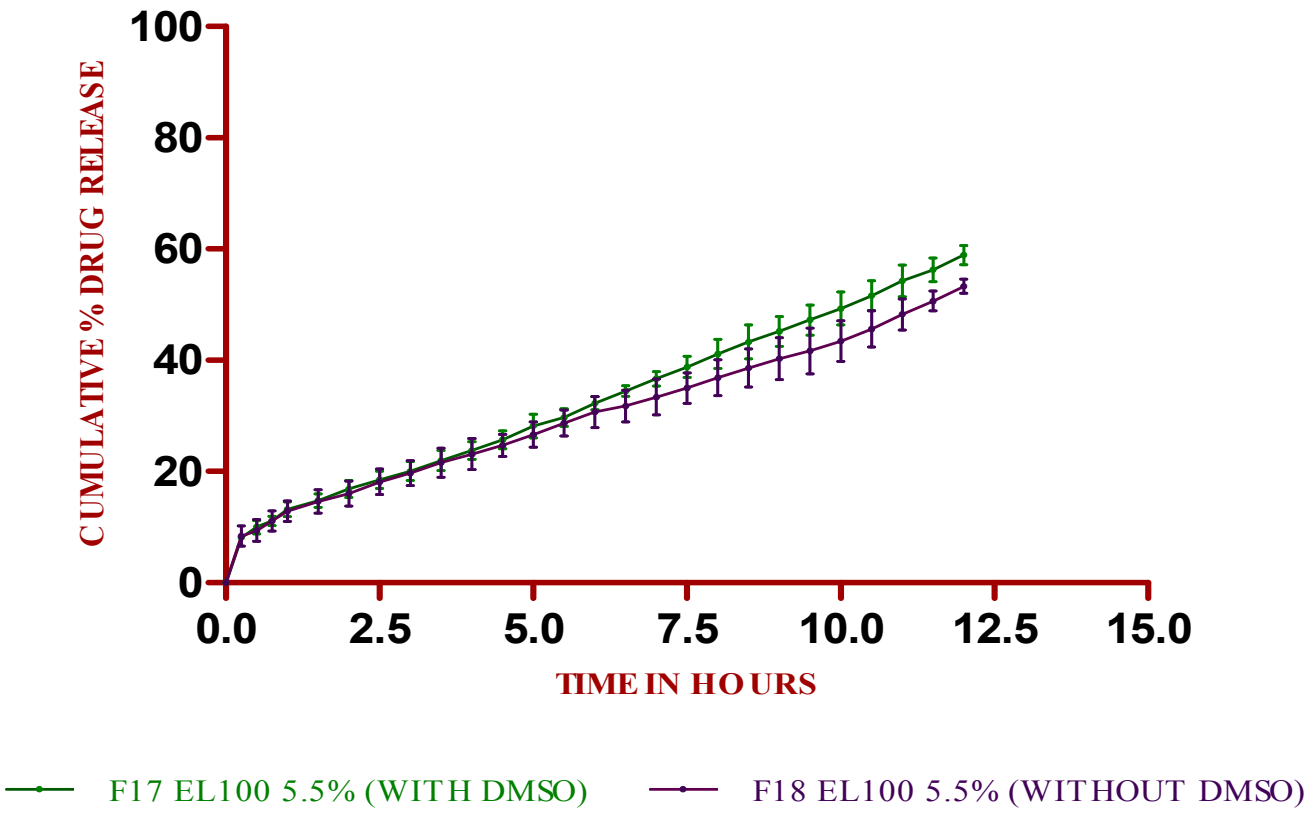


FIGURE 8I INVITRO RELEASE STUDY OF BENAZEPRIL HCL TRANSDERMAL PATCH CONTAINING EUDRAGIT L100 5.5% (WITH AND WITHOUT DMSO)

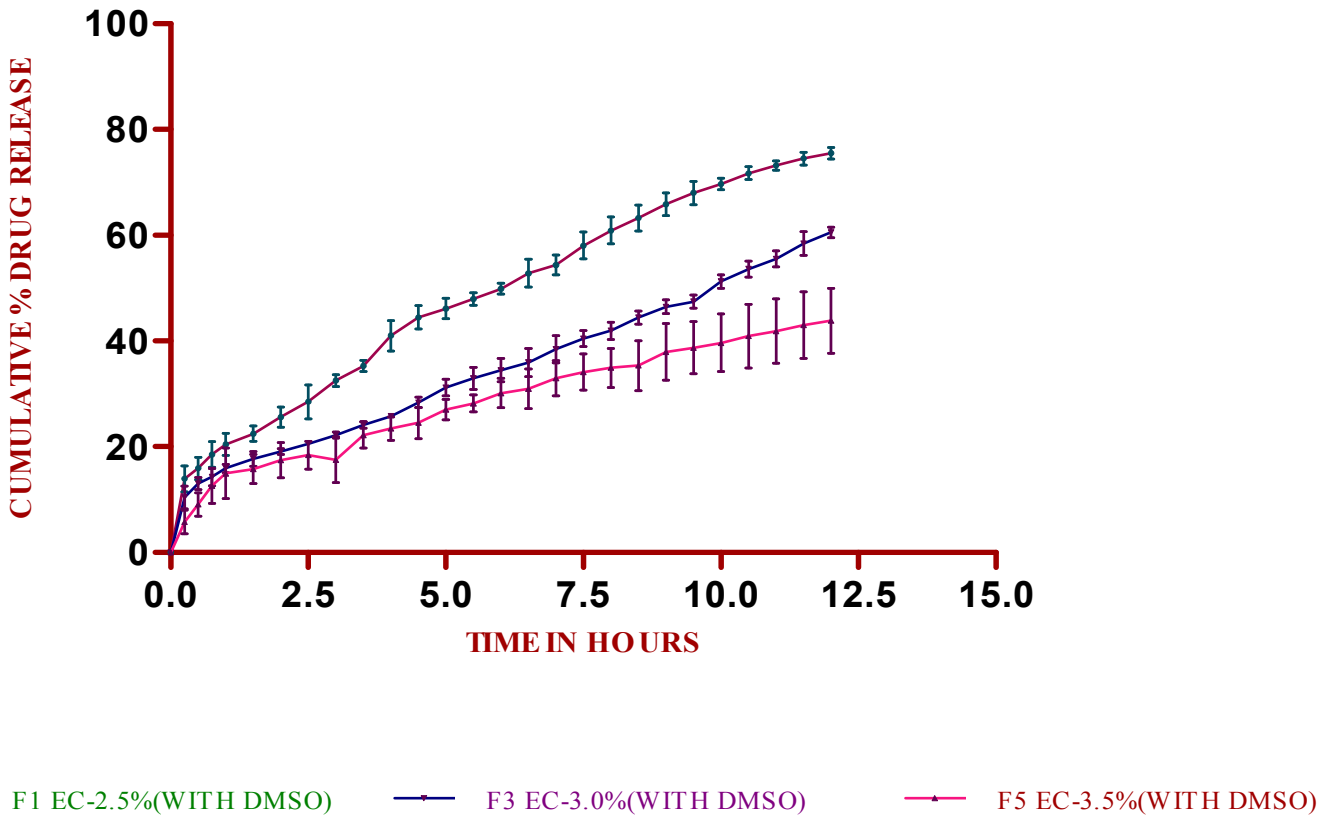


FIGURE 8J INVITRO RELEASE STUDY OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH CONTAINING ETHYLCELLULOSE AT VARIOUS CONCENTRATIONS (WITH PERMEATION ENHANCER)

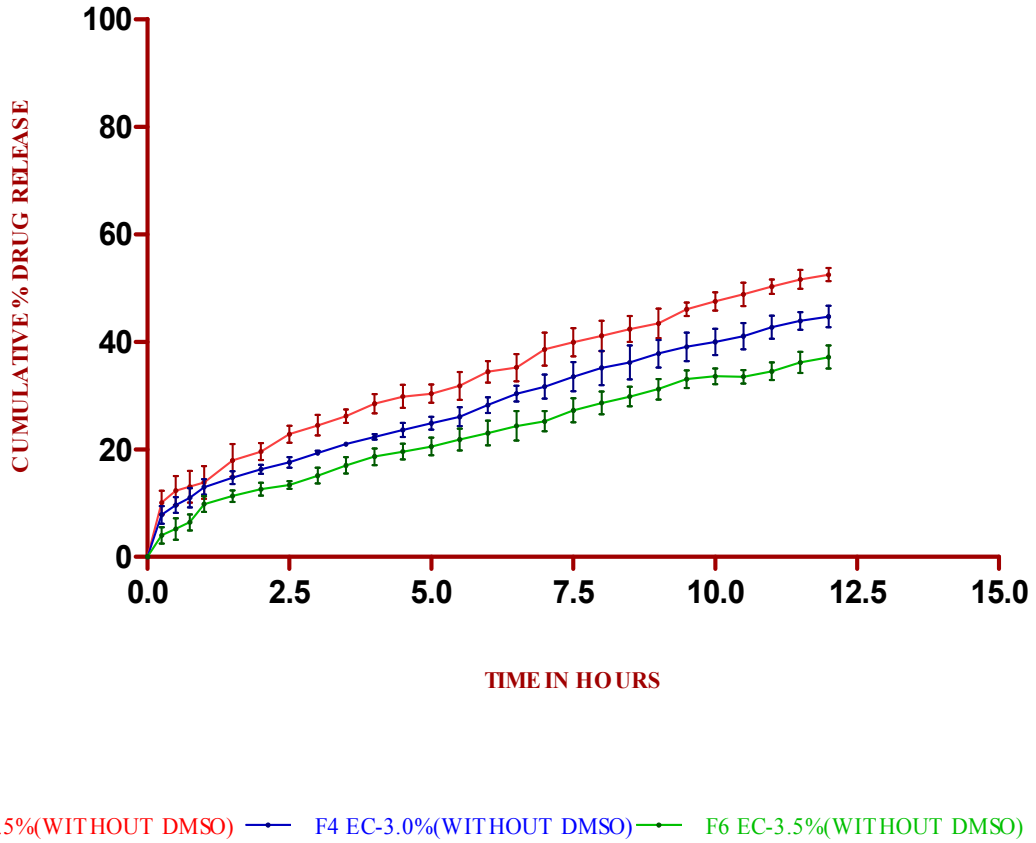


FIGURE 8K INVITRO RELEASE STUDY OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH CONTAINING ETHYLCELLULOSE AT VARIOUS CONCENTRATIONS (WITHOUT PERMEATION ENHANCER)

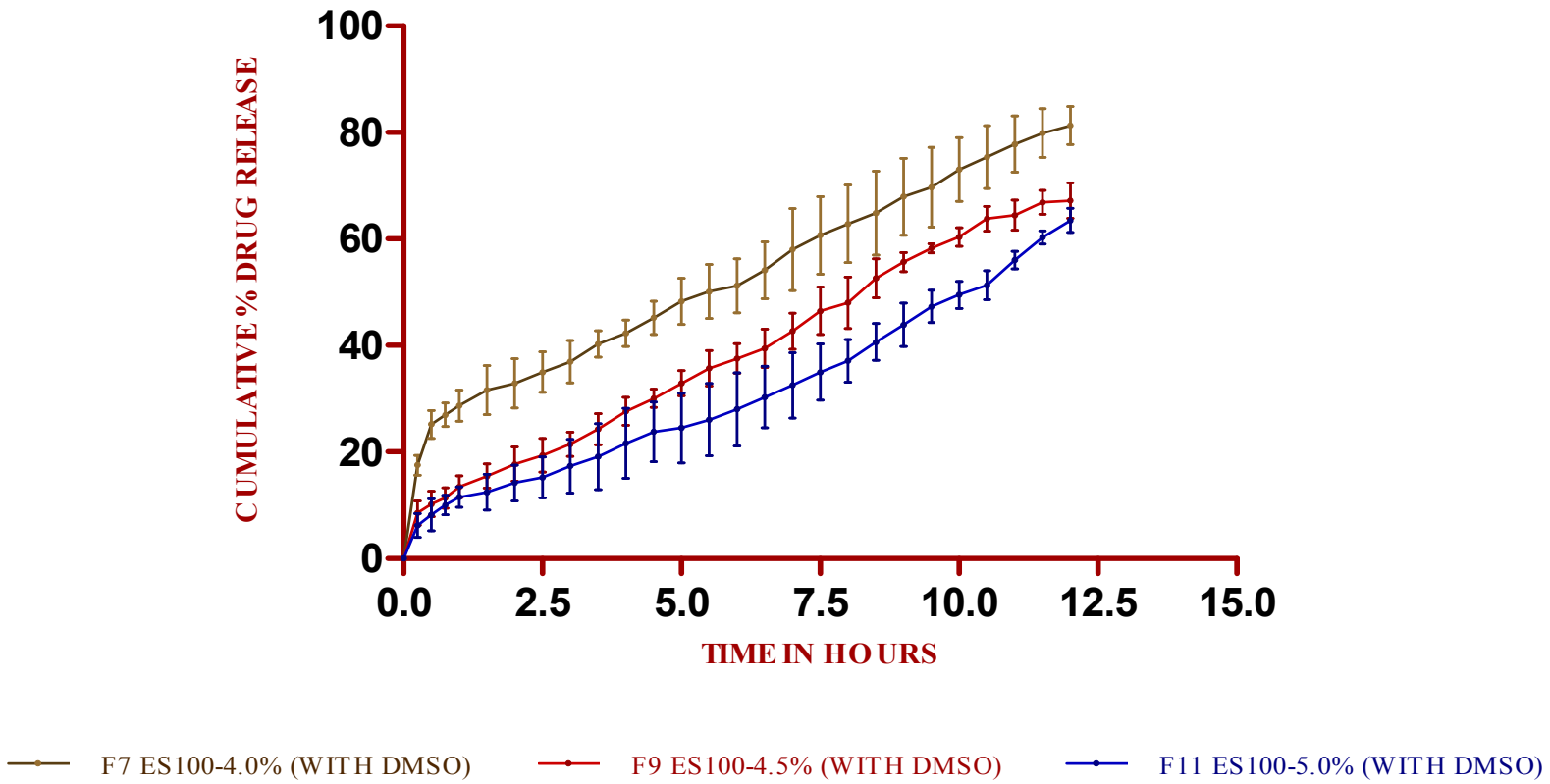


FIGURE 8L INVITRO RELEASE STUDY OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH CONTAINING EUDRAGIT - S100 AT VARIOUS CONCENTRATIONS (WITH PERMEATION ENHANCER)

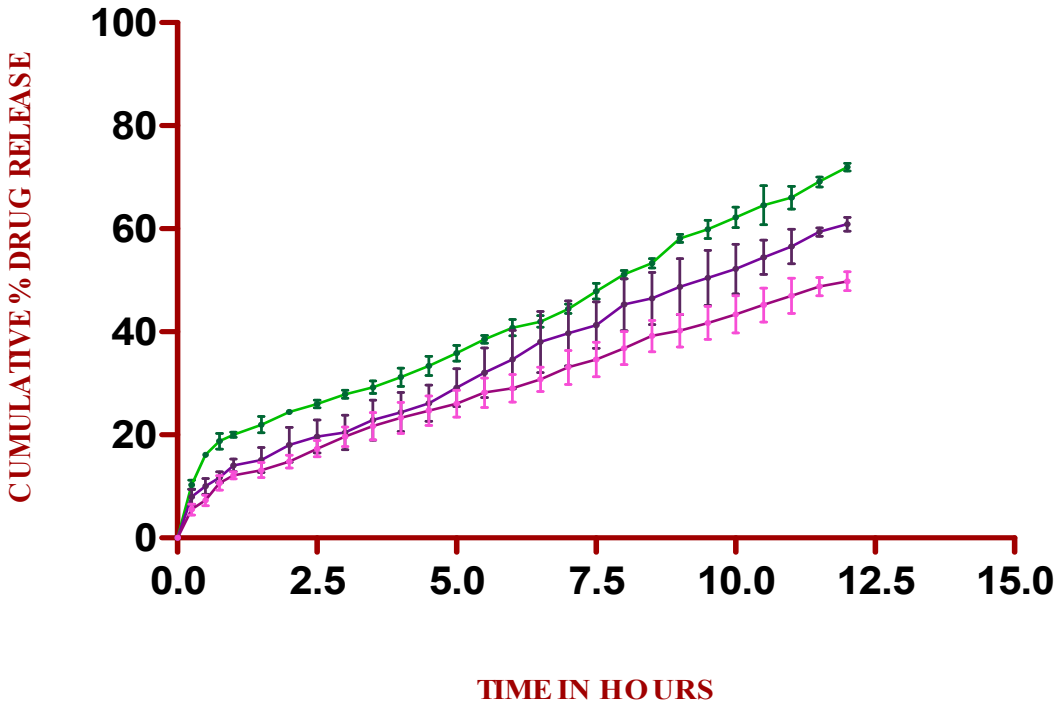


FIGURE 8M INVITRO RELEASE STUDY OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH CONTAINING EUDRAGIT - S100 AT VARIOUS CONCENTRATIONS (WITHOUT PERMEATION ENHANCER)

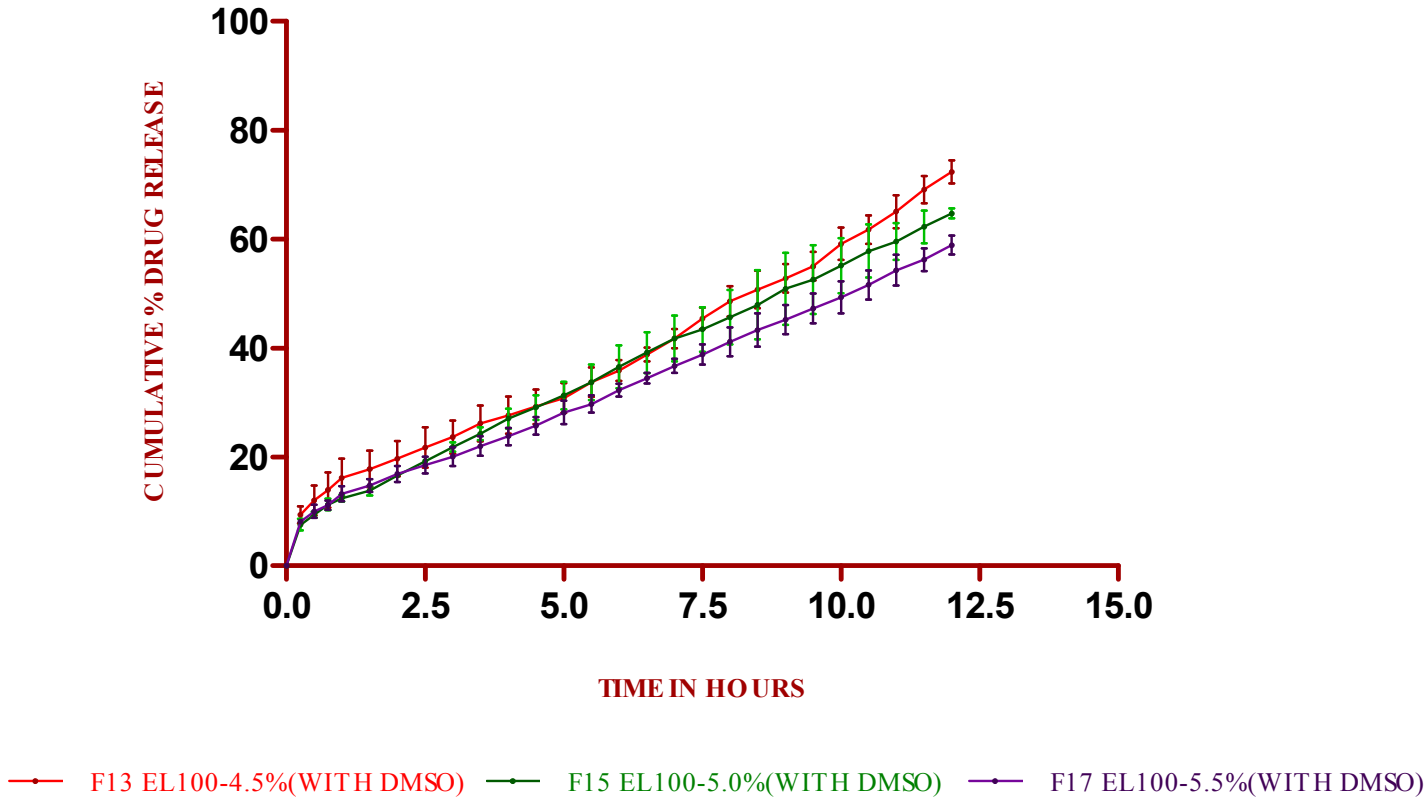


FIGURE 8N INVITRO RELEASE STUDY OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH CONTAINING EUDRAGIT – L100 AT VARIOUS CONCENTRATIONS (WITH PERMEATION ENHANCER)

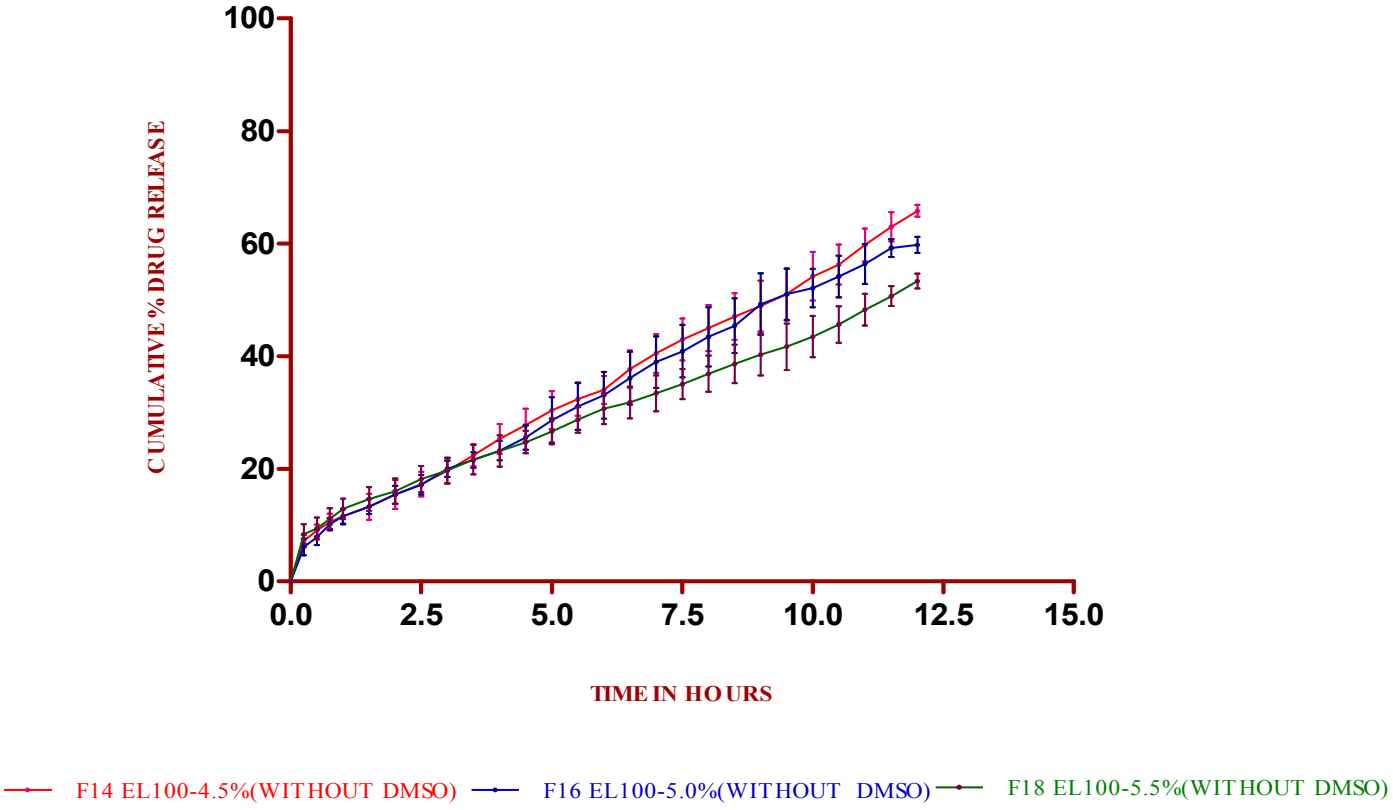


FIGURE 8O INVITRO RELEASE STUDY OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH CONTAINING EUDRAGIT – L100 AT VARIOUS CONCENTRATIONS (WITHOUT PERMEATION ENHANCER)

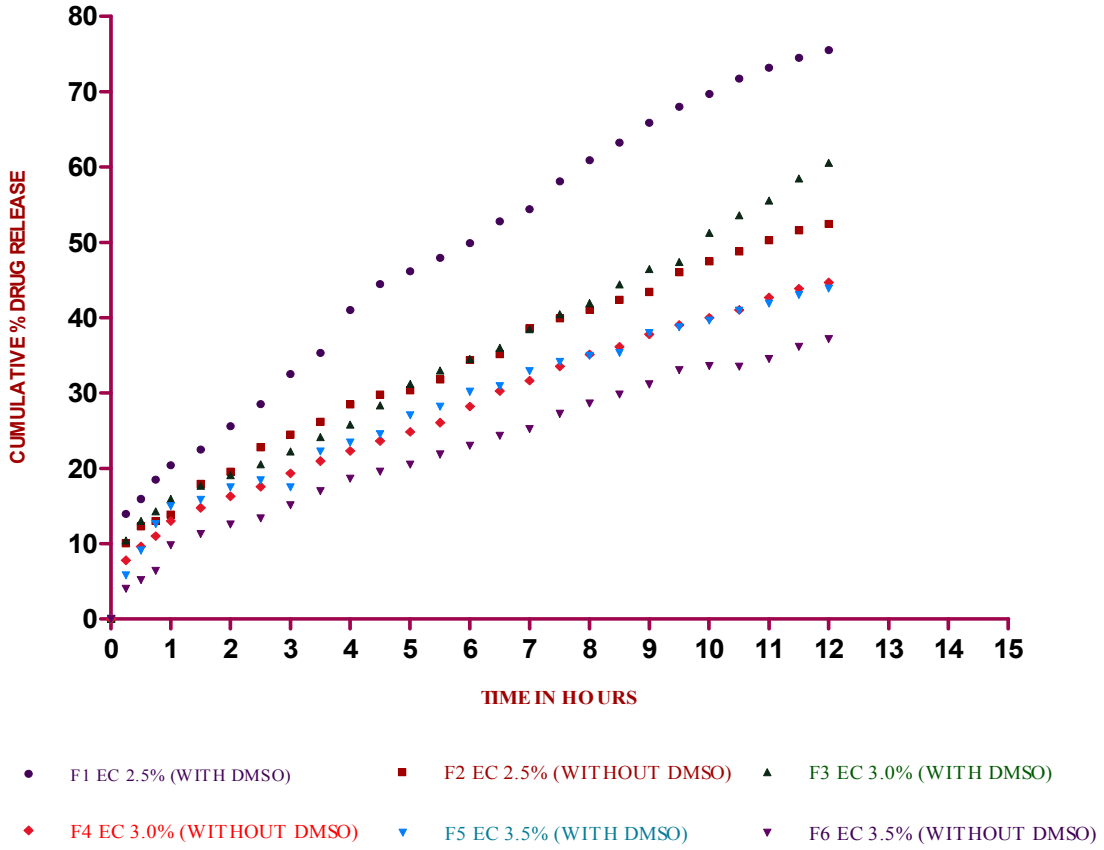


FIGURE 9A COMPARISON OF ZERO ORDER RELEASE KINETICS OF THE FORMULATIONS CONTAINING ETHYLCELLULOSE AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

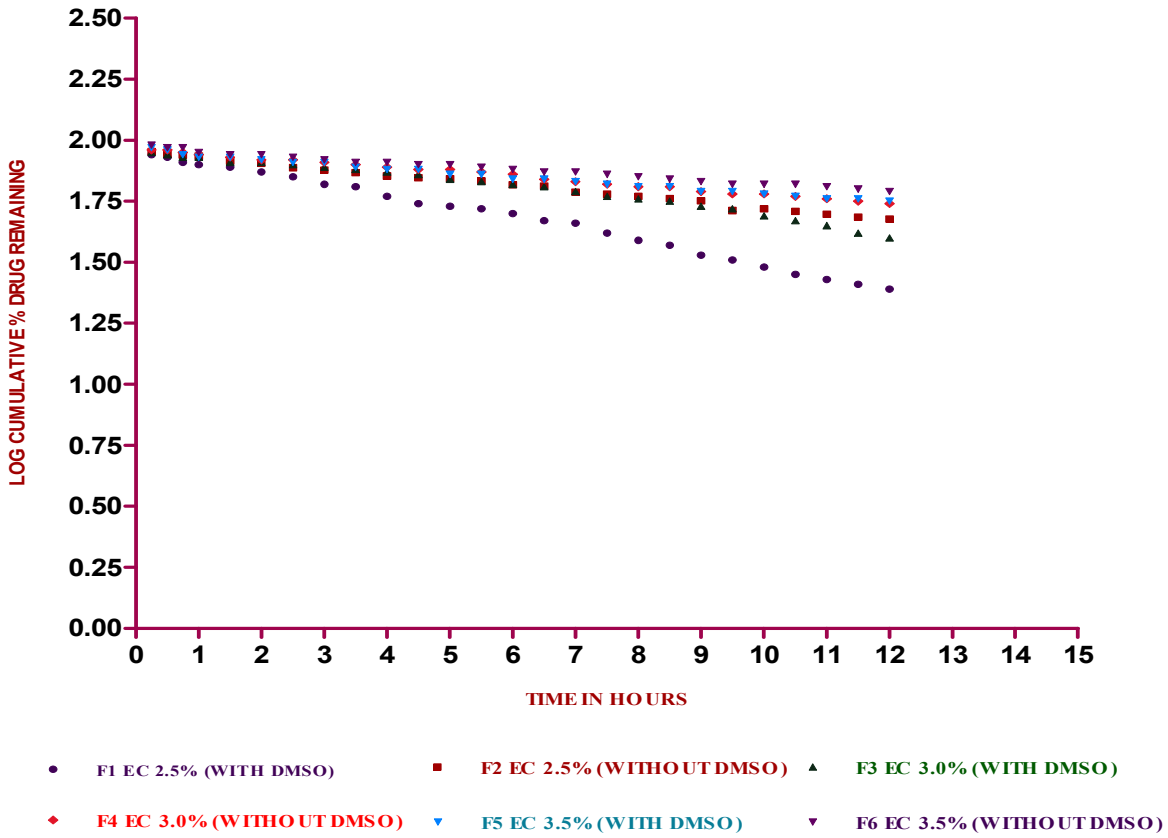


FIGURE 9B COMPARISON OF FIRST ORDER RELEASE KINETICS OF THE FORMULATIONS CONTAINING ETHYLCELLULOSE AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

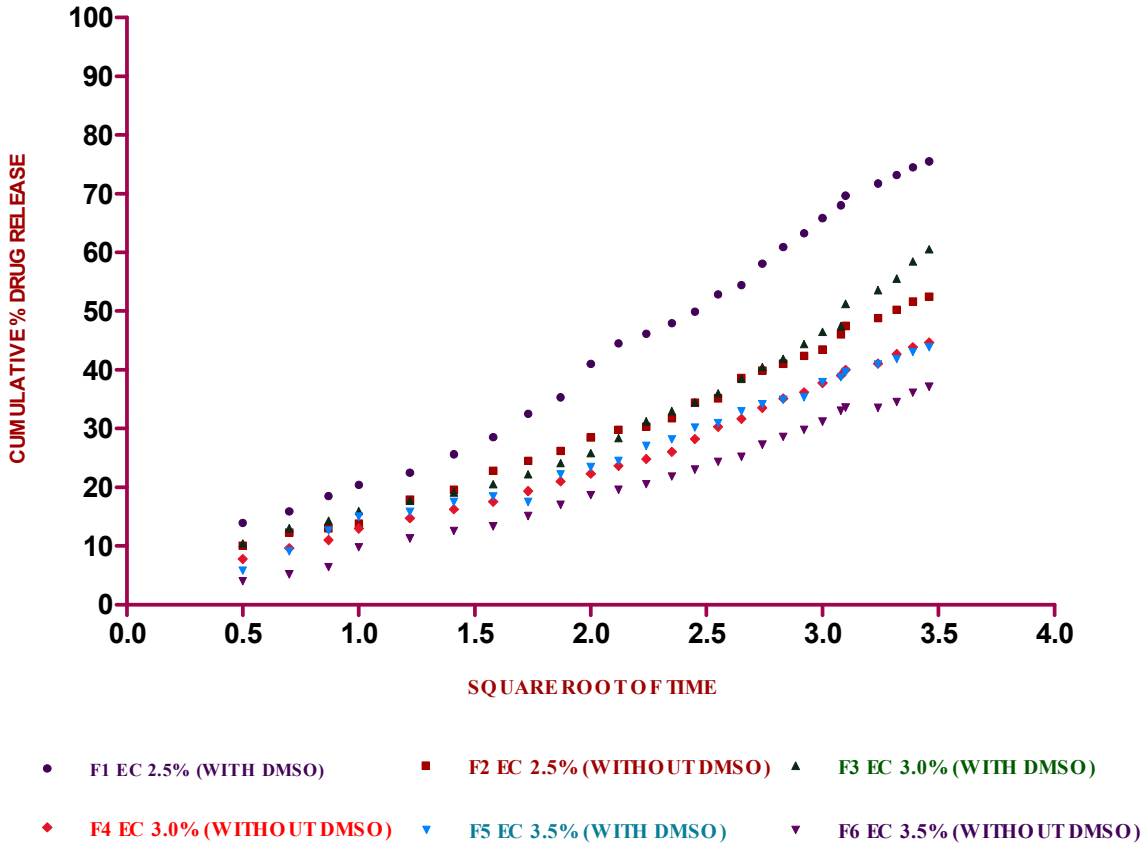


FIGURE 9C COMPARISON OF HIGUCHI RELEASE KINETICS OF THE FORMULATIONS CONTAINING ETHYLCELLULOSE AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

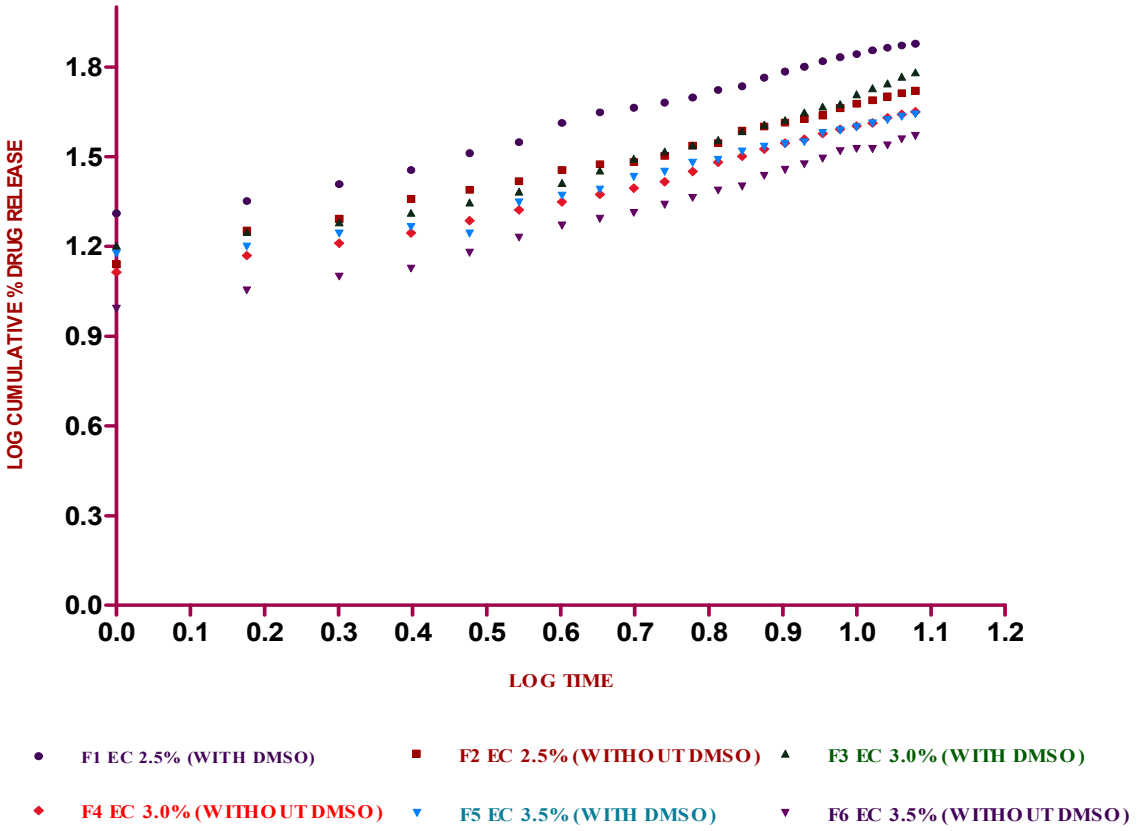


FIGURE 9D COMPARISON OF KORSMEYER AND PEPPAS RELEASE KINETICS OF THE FORMULATIONS CONTAINING ETHYLCELLULOSE AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

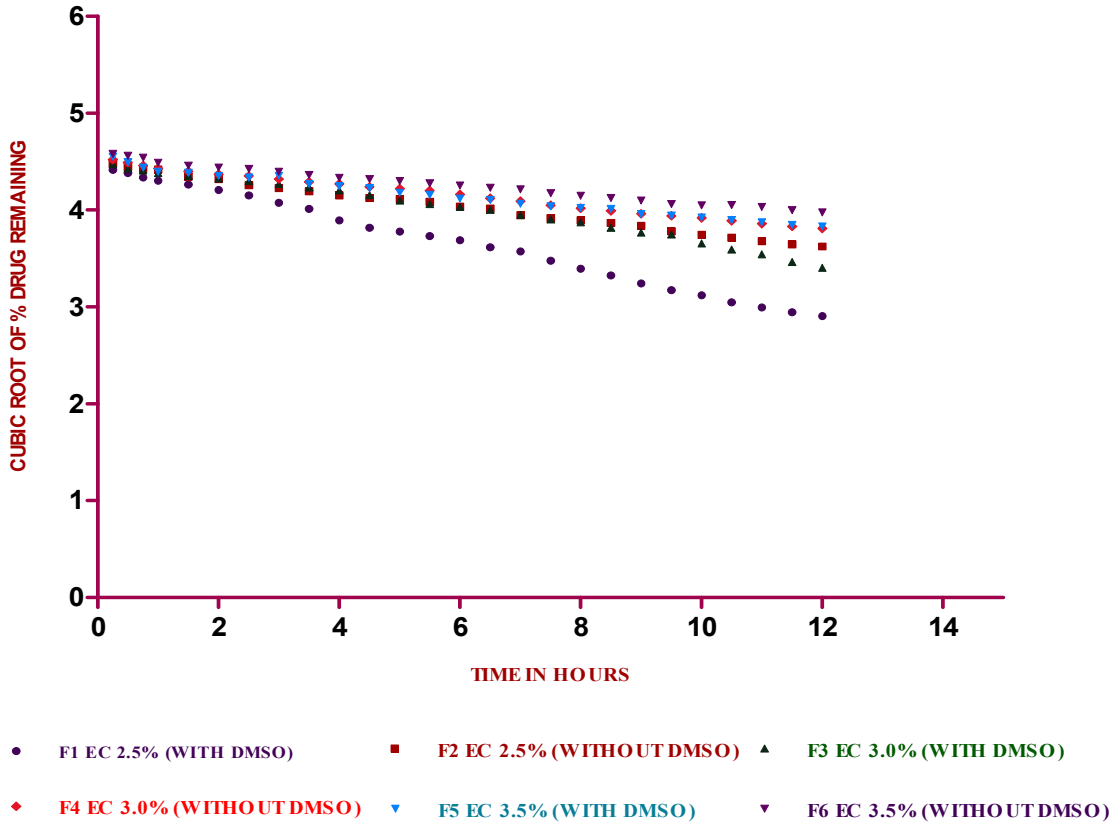


FIGURE 9E COMPARISON OF HIXSON-CROWELL RELEASE KINETICS OF THE FORMULATIONS CONTAINING ETHYLCELLULOSE AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

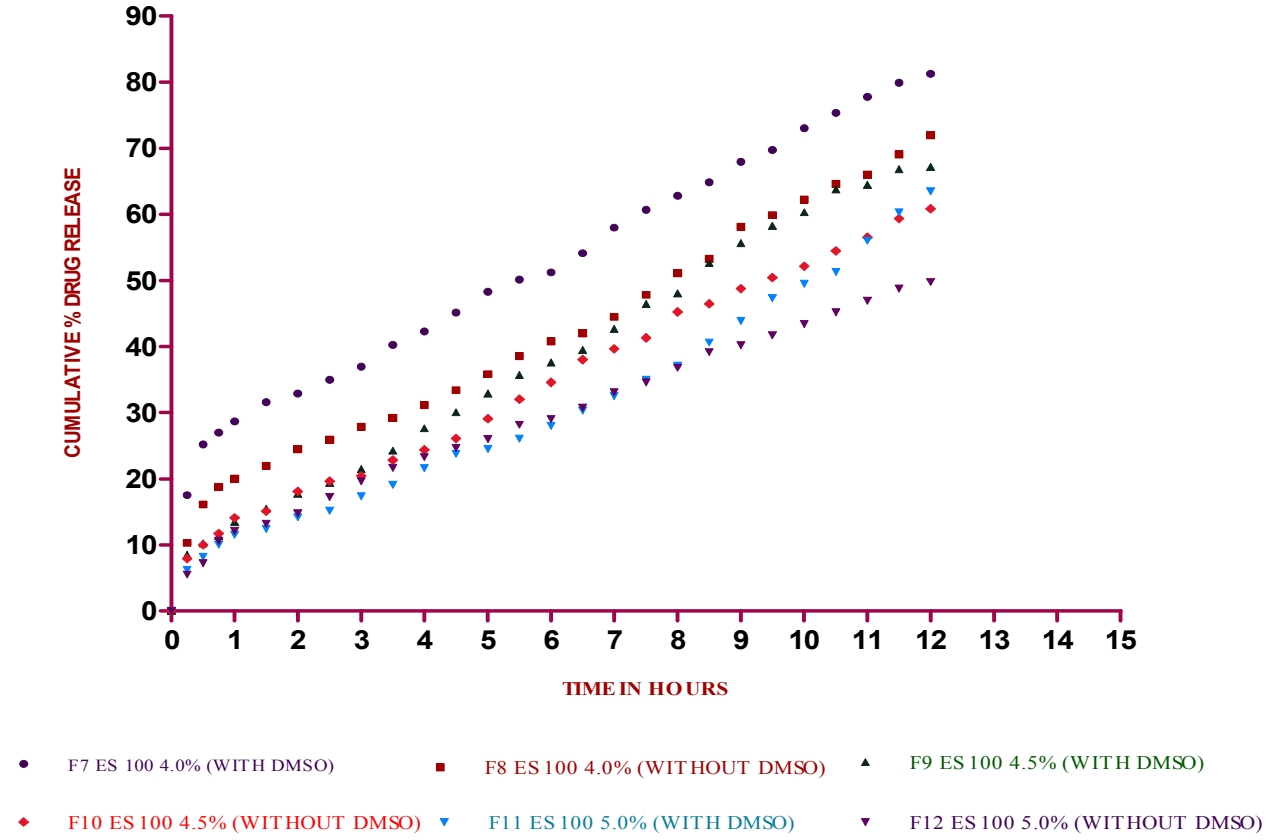


FIGURE 9F COMPARISON OF ZERO ORDER RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT S 100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

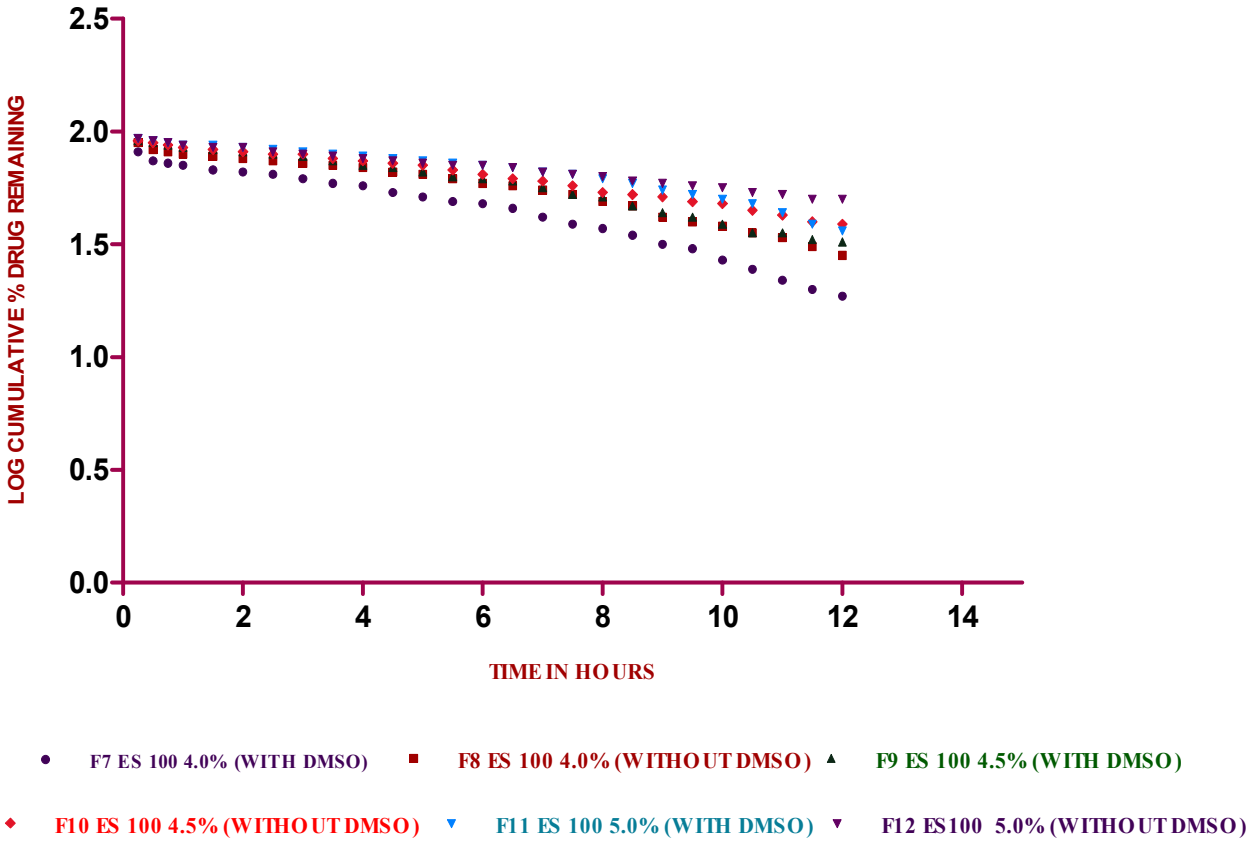


FIGURE 9G COMPARISON OF FIRST ORDER RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT S100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

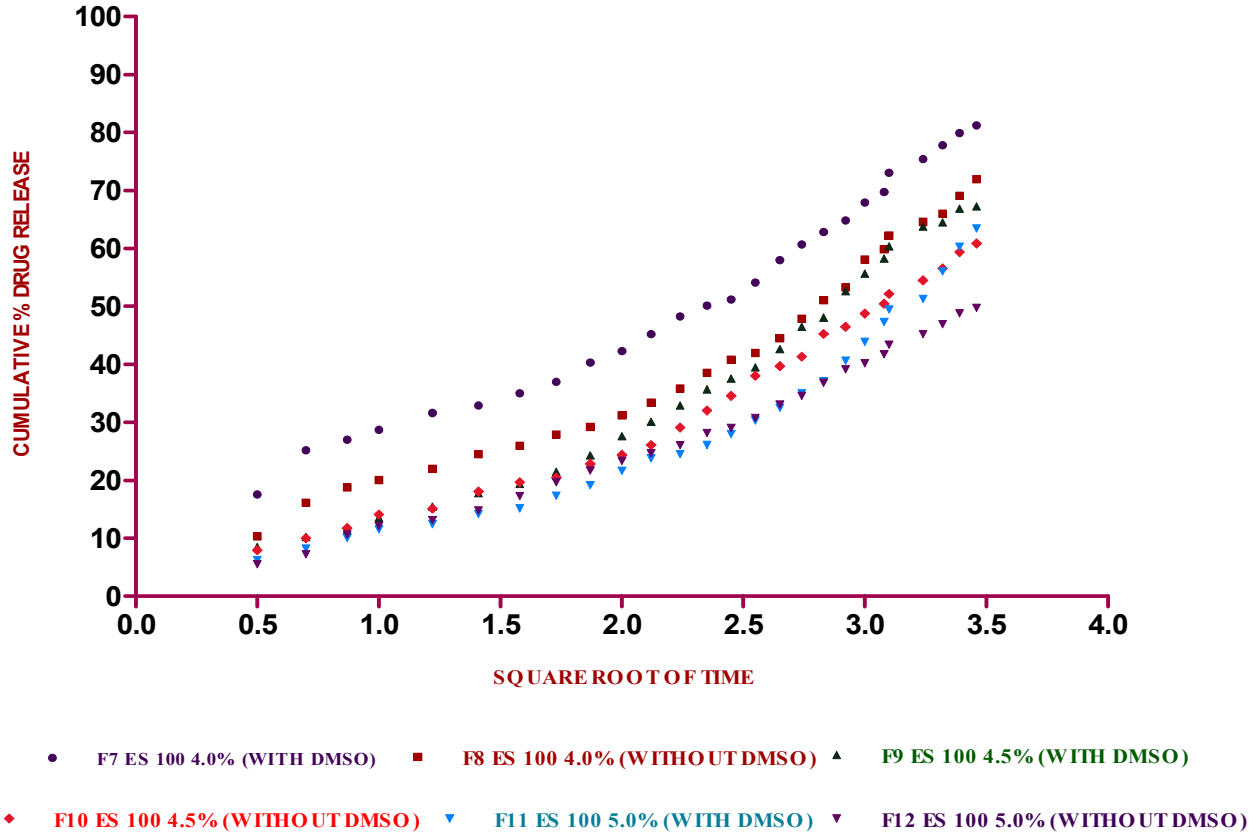


FIGURE 9H COMPARISON OF HIGUCHI RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT S 100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

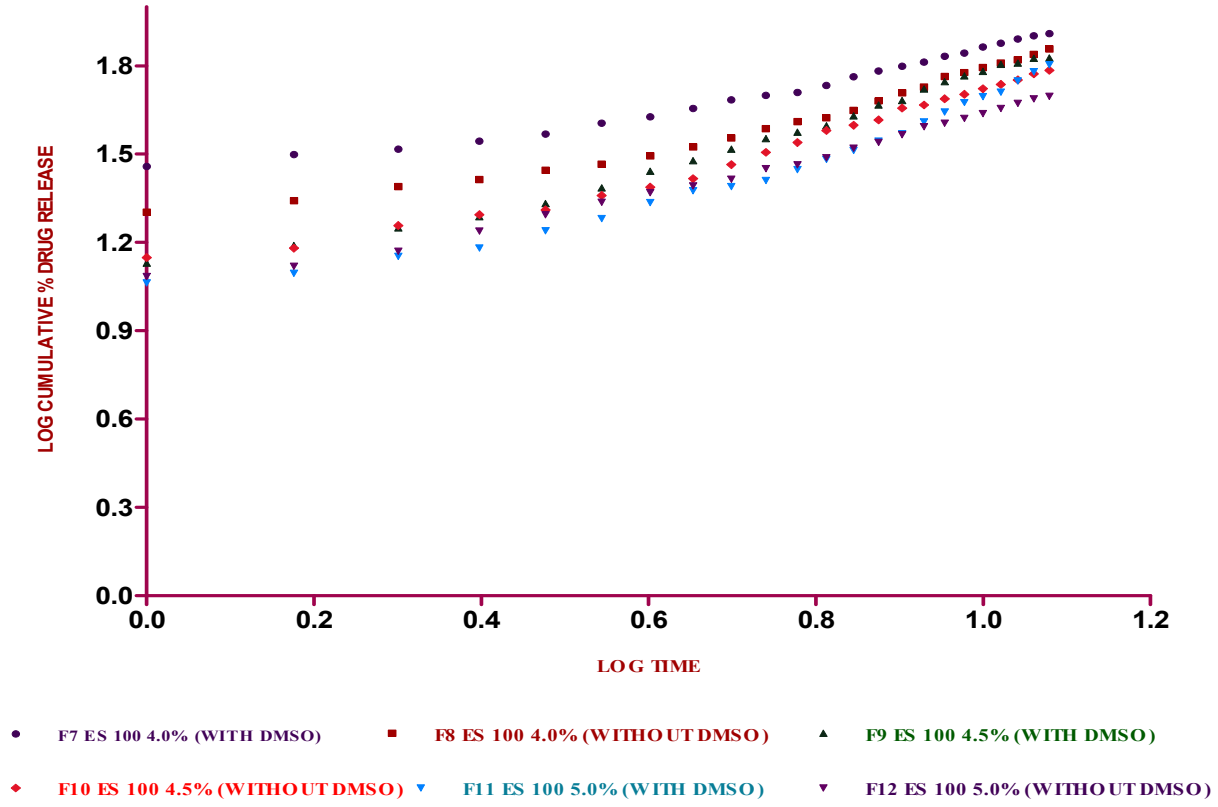


FIGURE 9I COMPARISON OF KORSMEYER AND PEPPAS RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT S 100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

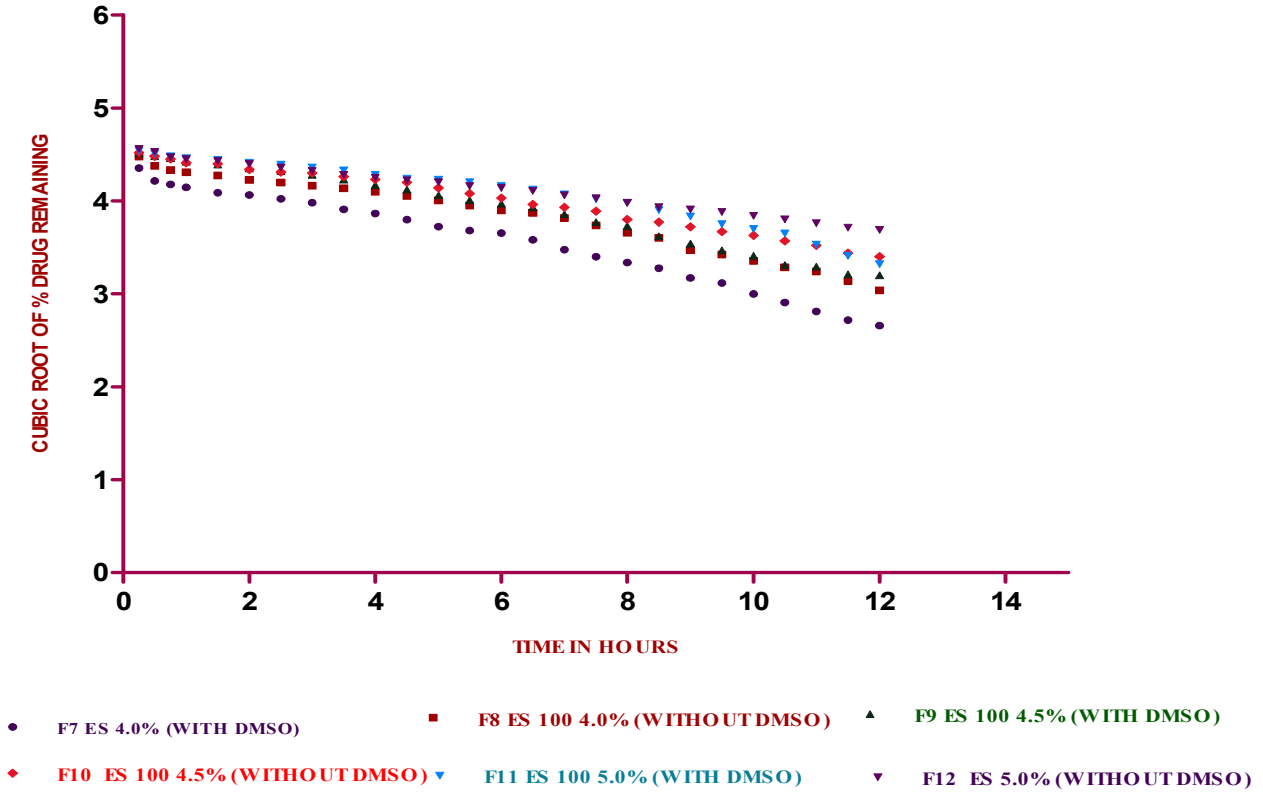


FIGURE 9J COMPARISON OF HIXSON-CROWELL RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT S 100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

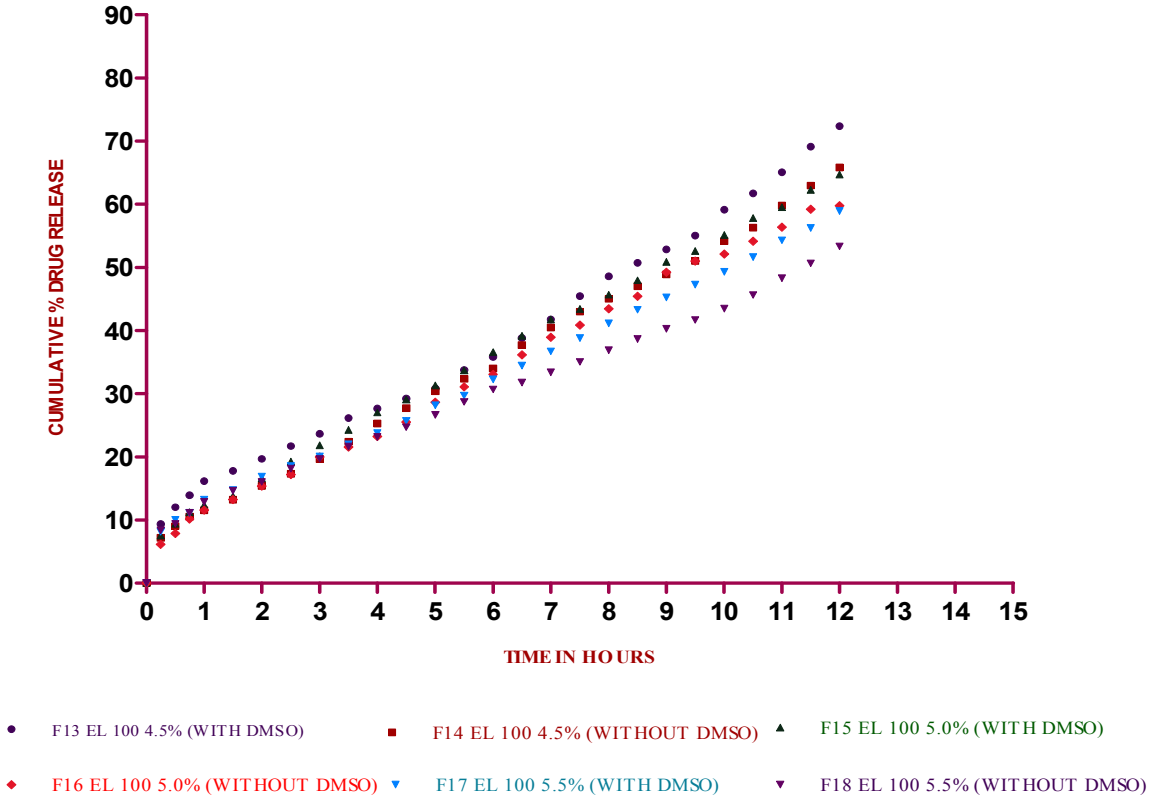


FIGURE 9K COMPARISON OF ZERO ORDER RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT L 100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

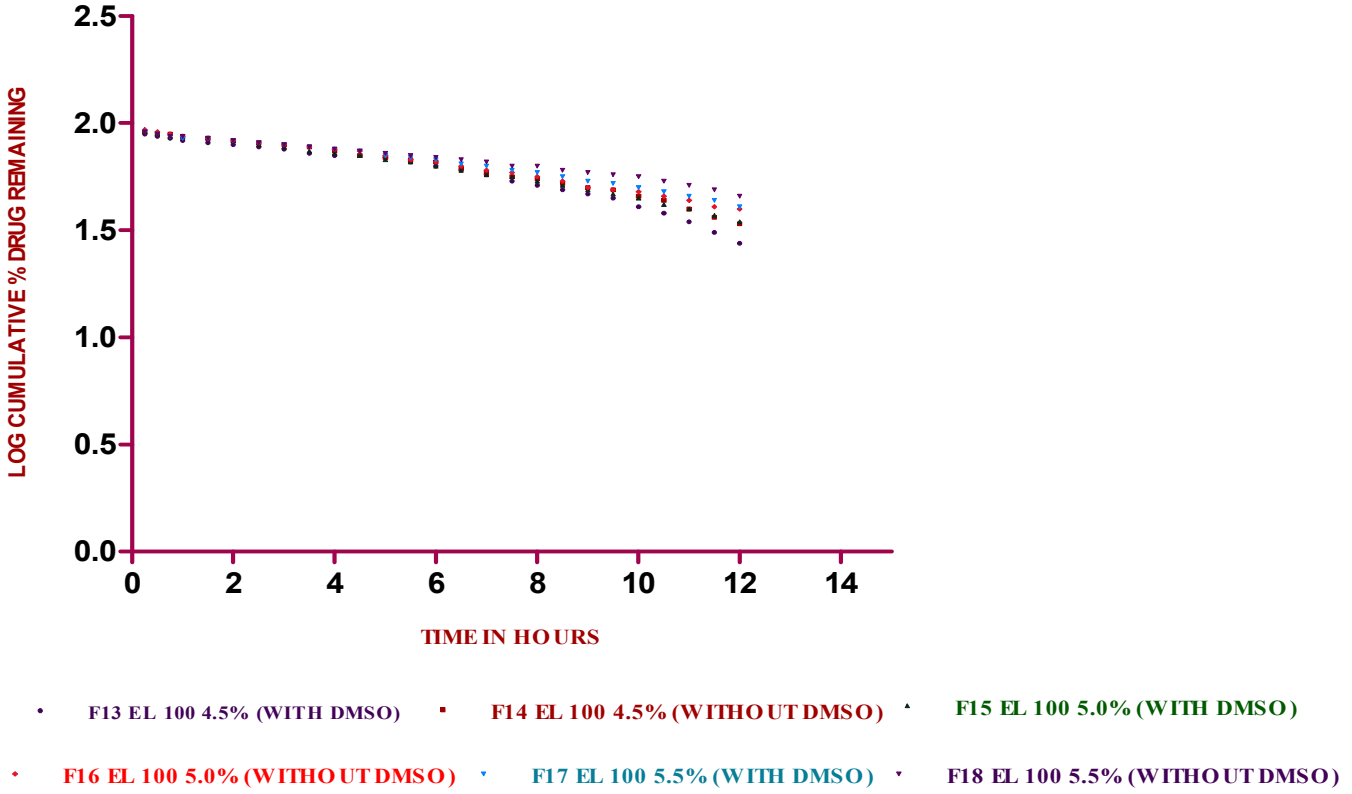


FIGURE 9L COMPARISON OF FIRST ORDER RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT L 100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

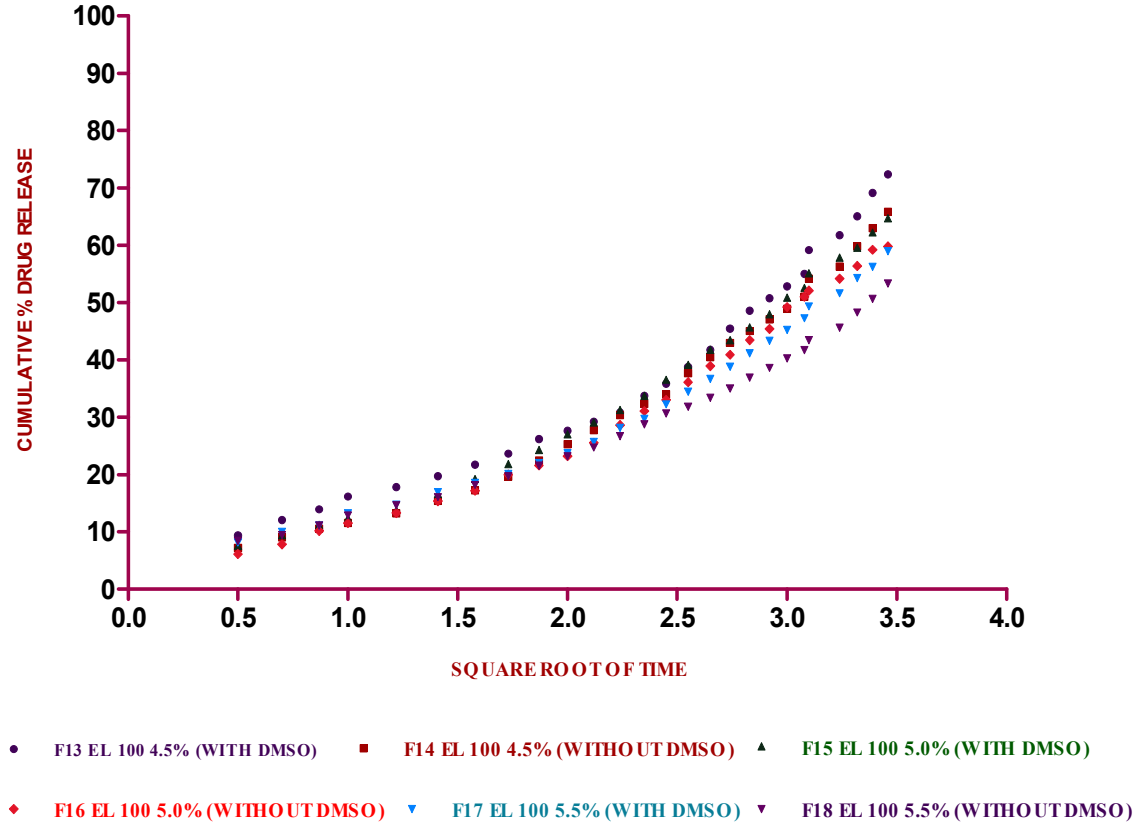


FIGURE 9M COMPARISON OF HIGUCHI RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT L 100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

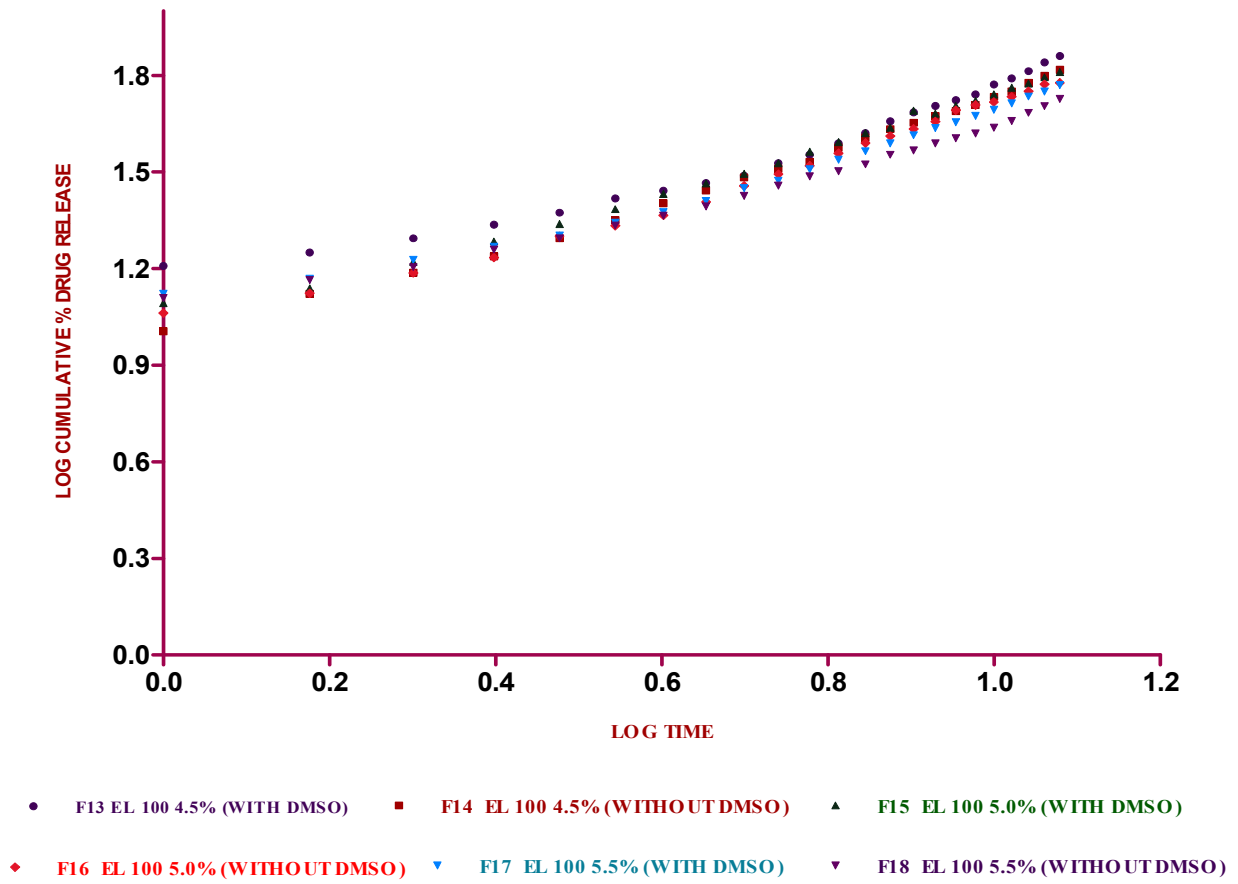


FIGURE 9N COMPARISON OF KORSMEYER AND PEPPAS RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT L 100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

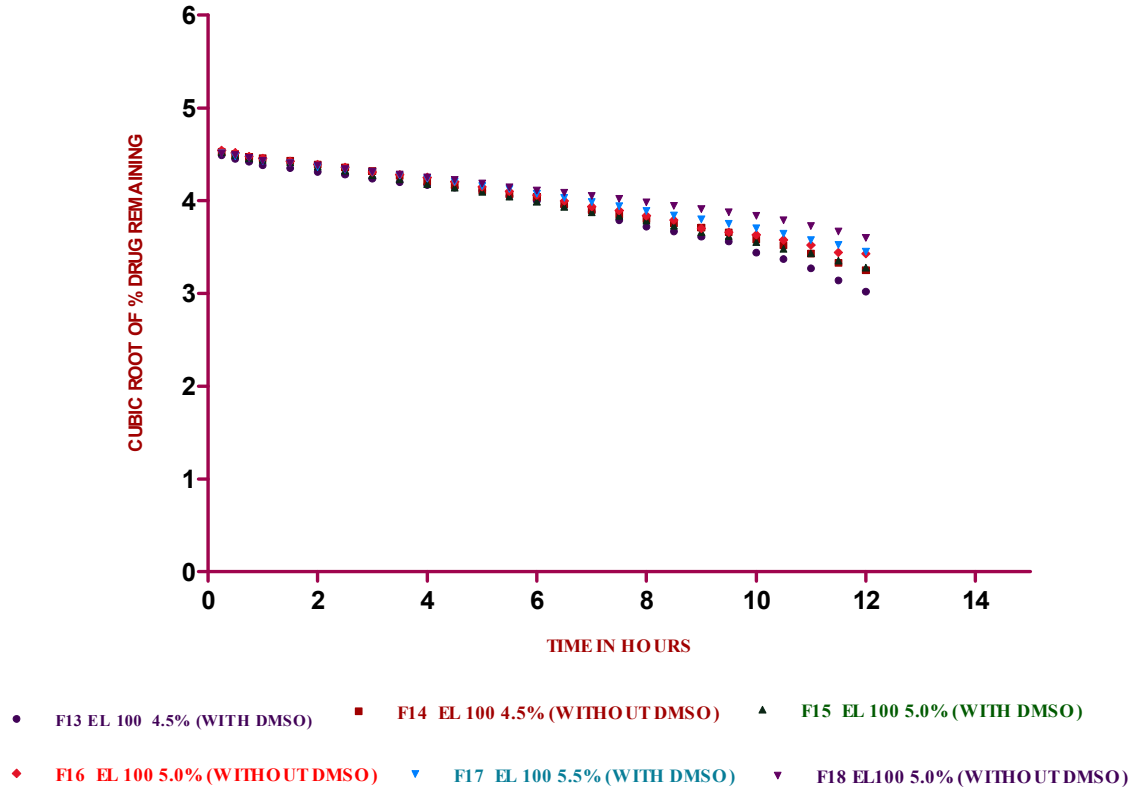


FIGURE 9O COMPARISON OF HIXSON-CROWELL RELEASE KINETICS OF THE FORMULATIONS CONTAINING EUDRAGIT L 100 AT VARIOUS CONCENTRATIONS (WITH AND WITHOUT DIMETHYL SULFOXIDE)

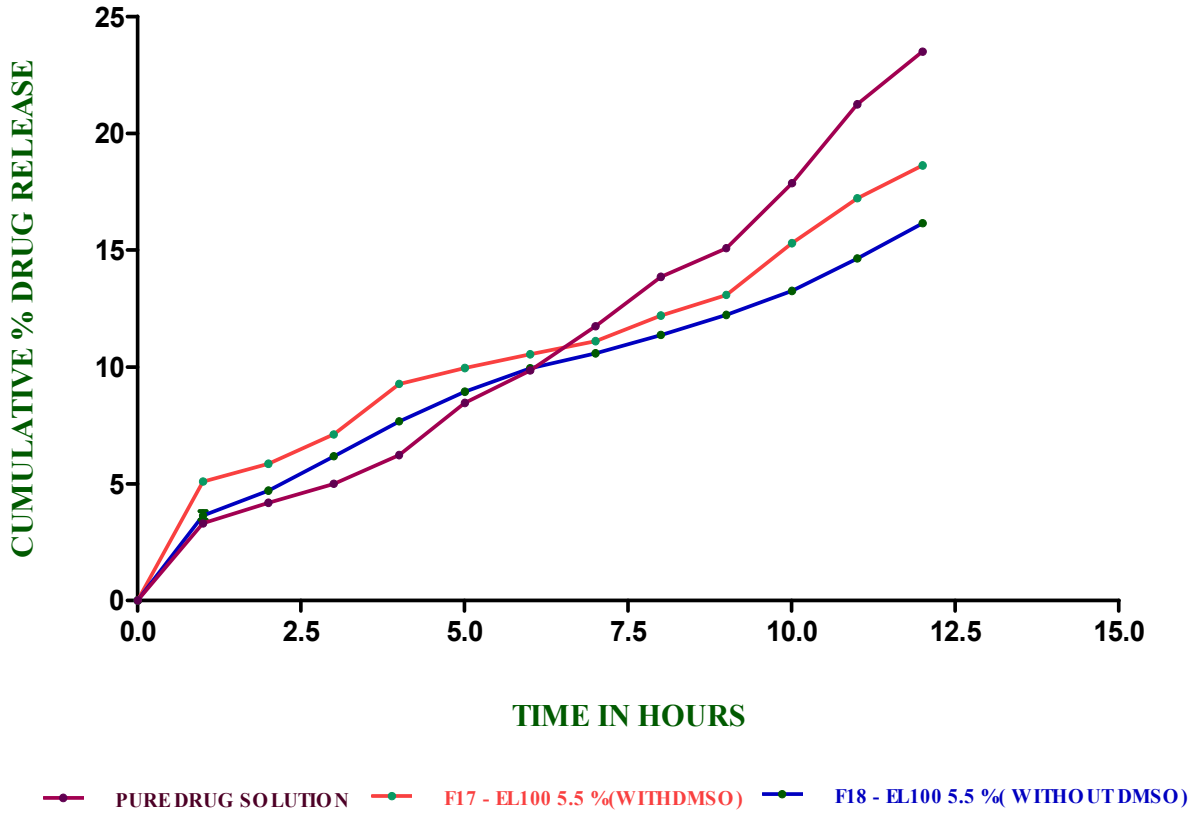


FIGURE 10A COMPARISION OF EXVIVO PERMEABILITY STUDY BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCHES WITH PURE DRUG SOLUTION

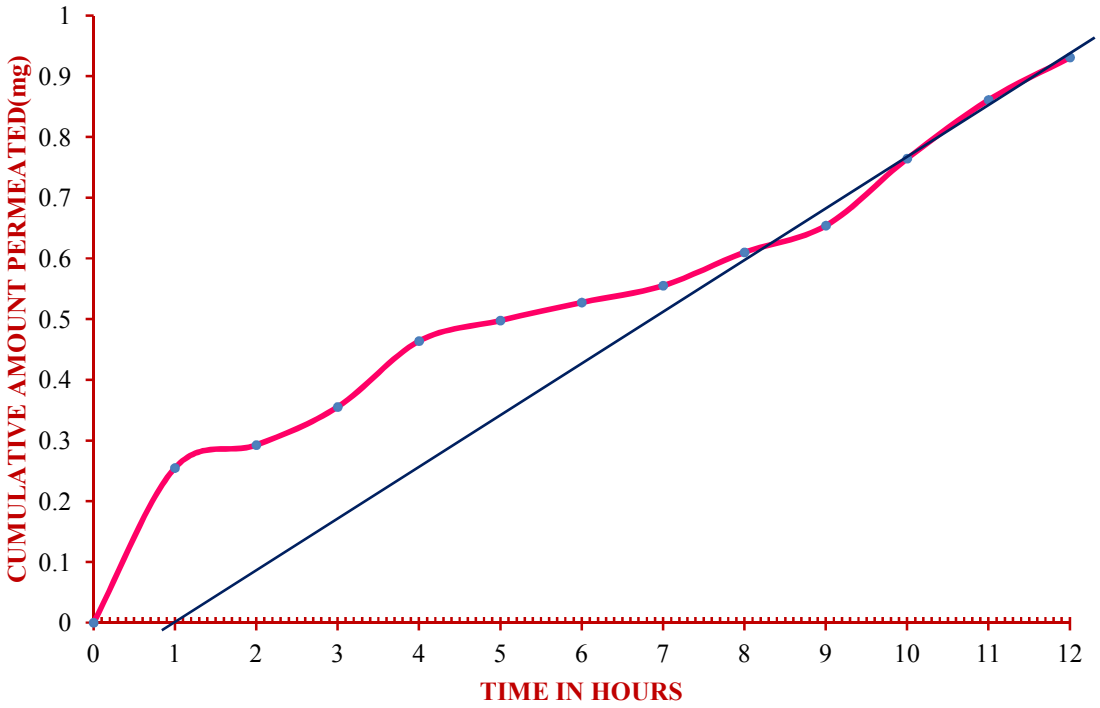


FIGURE 10B EXVIVO PERMEABILITY STUDY OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH CONTAINING EUDRAGIT L 100 5.5% (WITH DMSO)

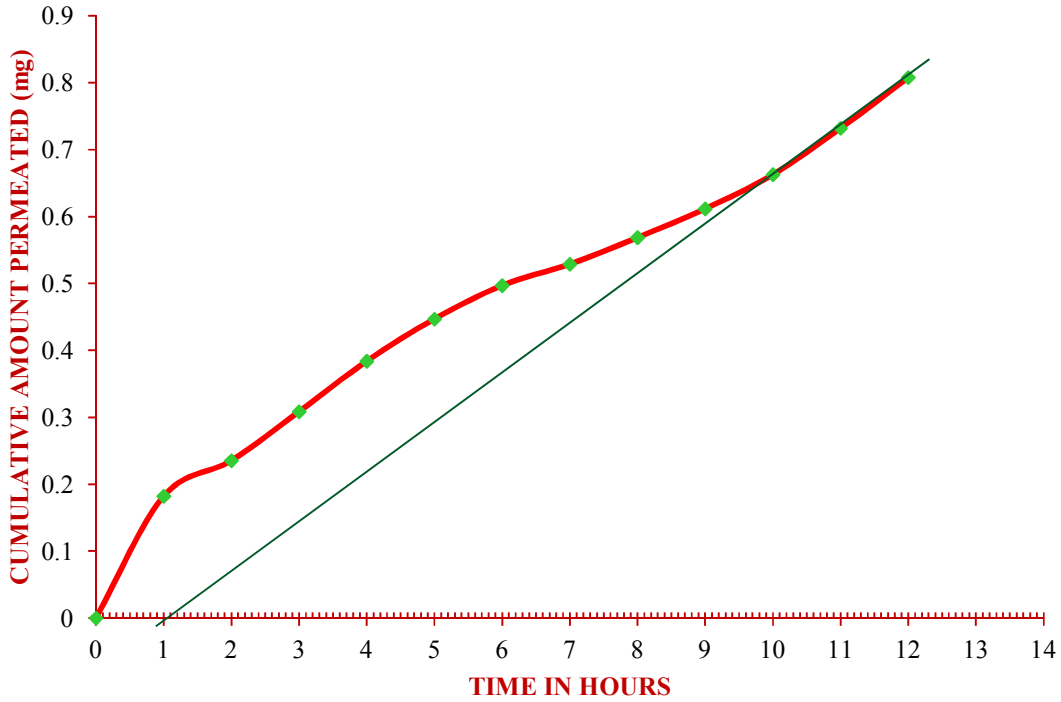


FIGURE 10C EXVIVO PERMEABILITY STUDY OF BENAZEPRIL HYDROCHLORIDE TRANSDERMAL PATCH CONTAINING EUDRAGIT L 100 5.5% (WITH OUT DMSO)

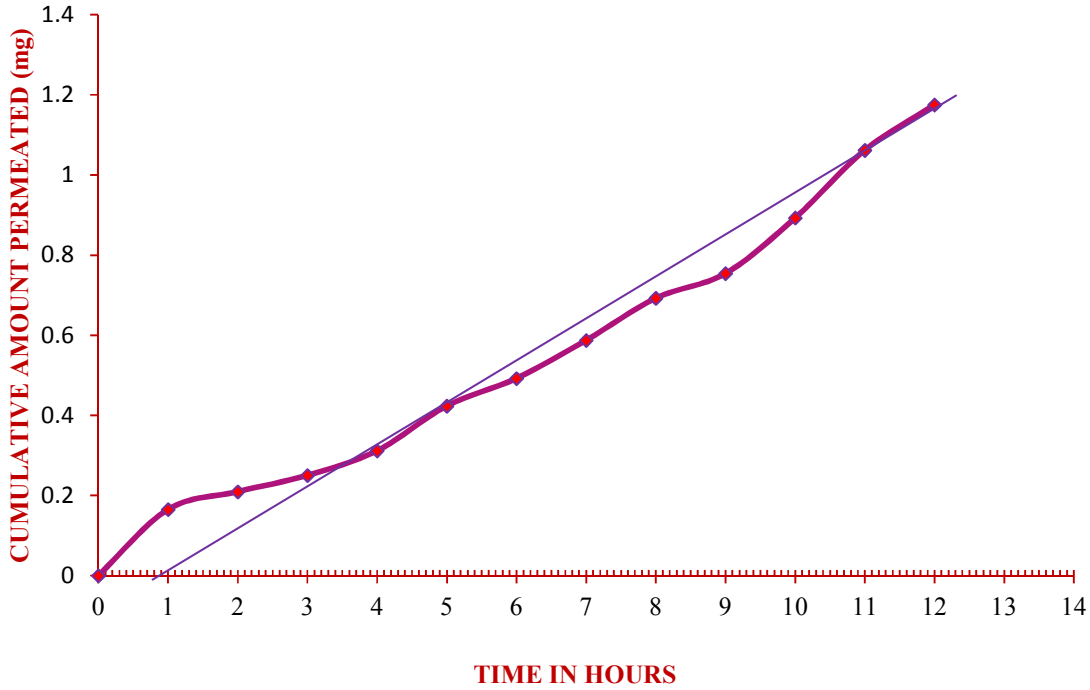


FIGURE 10D EXVIVO PERMEABILITY STUDY OF BENAZEPRIL HYDROCHLORIDE PURE DRUG SOLUTION

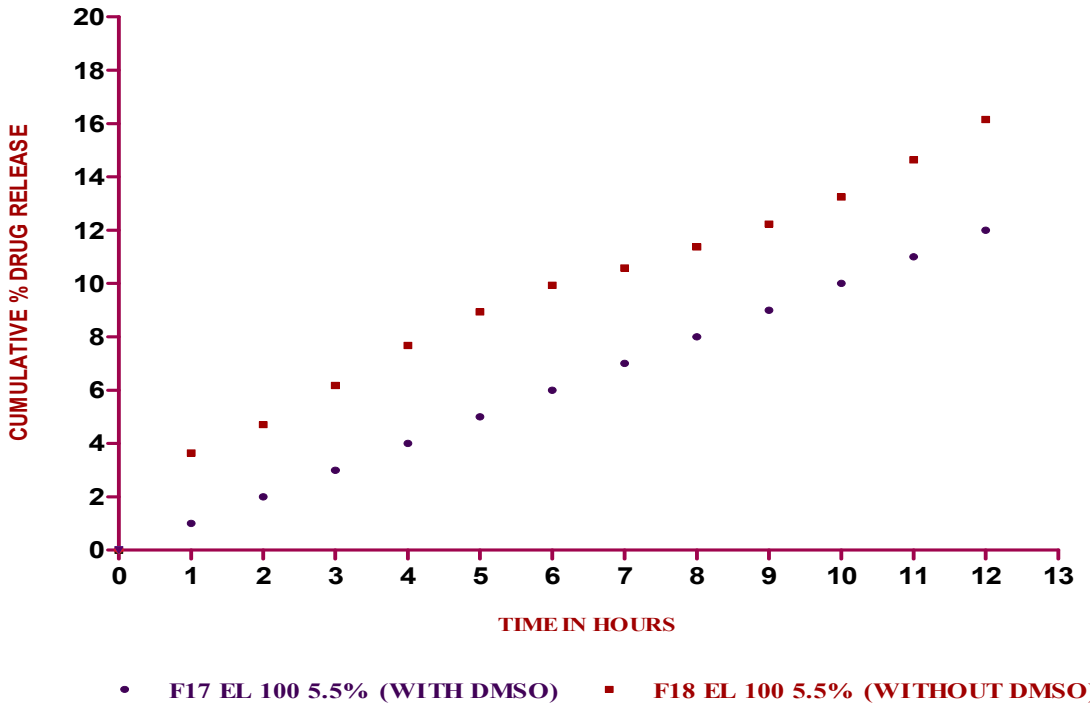


FIGURE 11A COMPARISON OF ZERO ORDER EXVIVO PERMEABILITY RELEASE KINETICS OF FORMULATIONS CONTAINING EUDRAGIT L 100 5.5% (WITH AND WITHOUT DMSO)

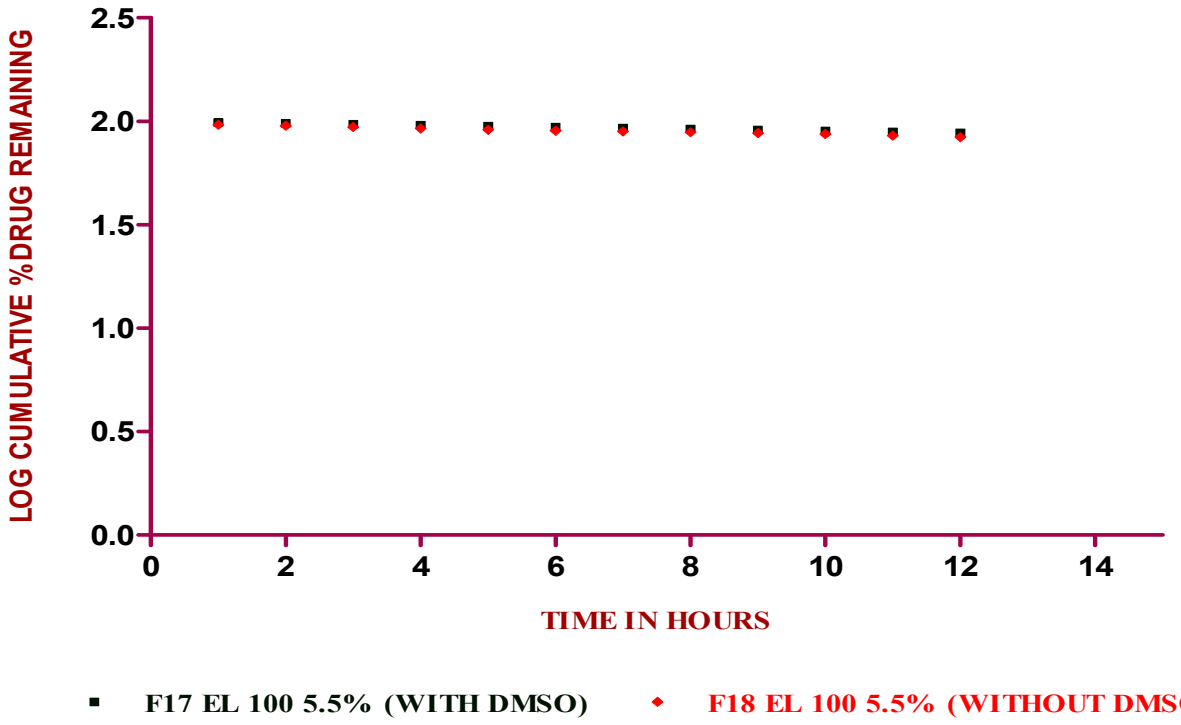


FIGURE 11B COMPARISON OF FIRST ORDER EXVIVO PERMEABILITY RELEASE KINETICS OF FORMULATIONS CONTAINING EUDRAGIT L 100 5.5% (WITH AND WITHOUT DMSO)

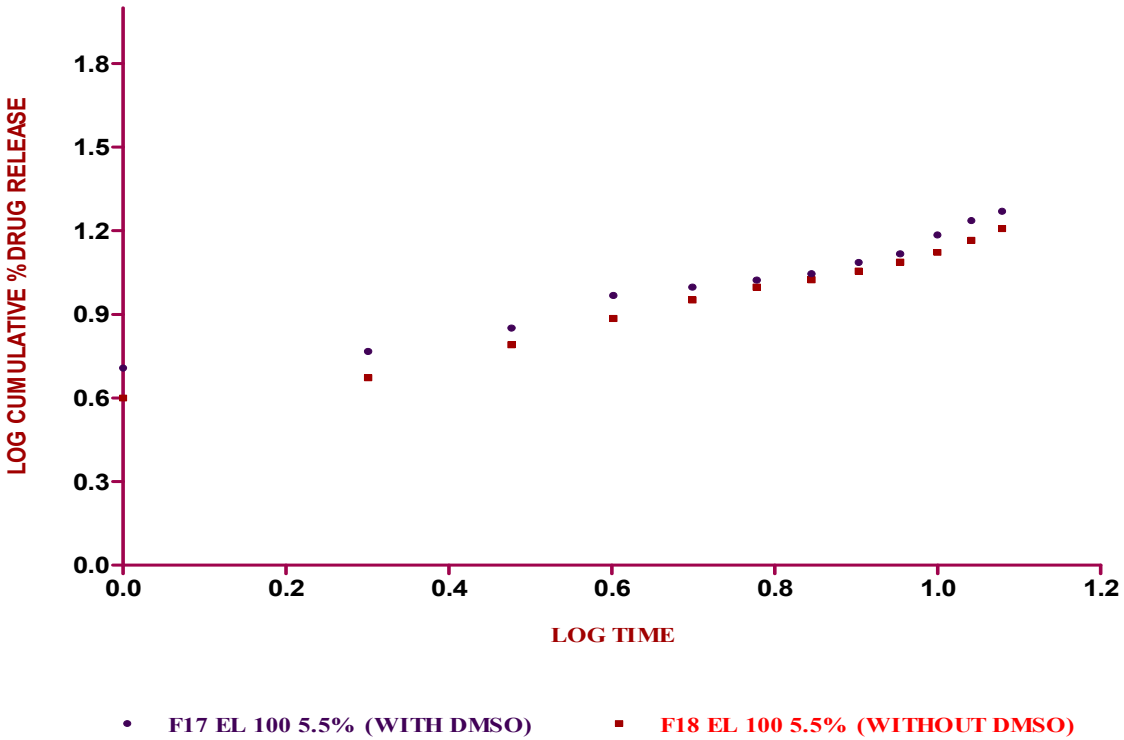


FIGURE 11C COMPARISON OF HIGUCHI EXVIVO PERMEABILITY RELEASE KINETICS OF FORMULATIONS CONTAINING EUDRAGIT L 100 5.5% (WITH AND WITHOUT DMSO)

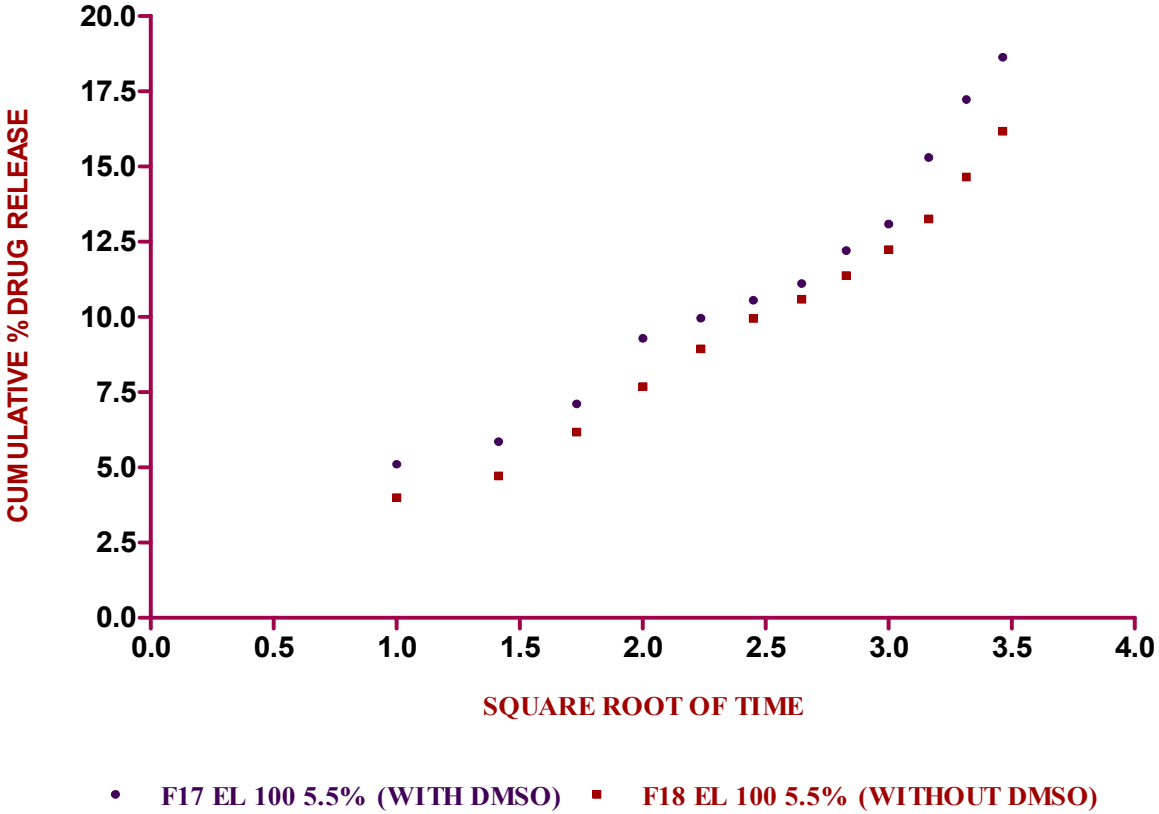


FIGURE 11D COMPARISON OF KORSMEYER AND PEPPAS EXVIVO PERMEABILITY RELEASE KINETICS OF FORMULATIONS CONTAINING EUDRAGIT L 100 5.5% (WITH AND WITHOUT DMSO)

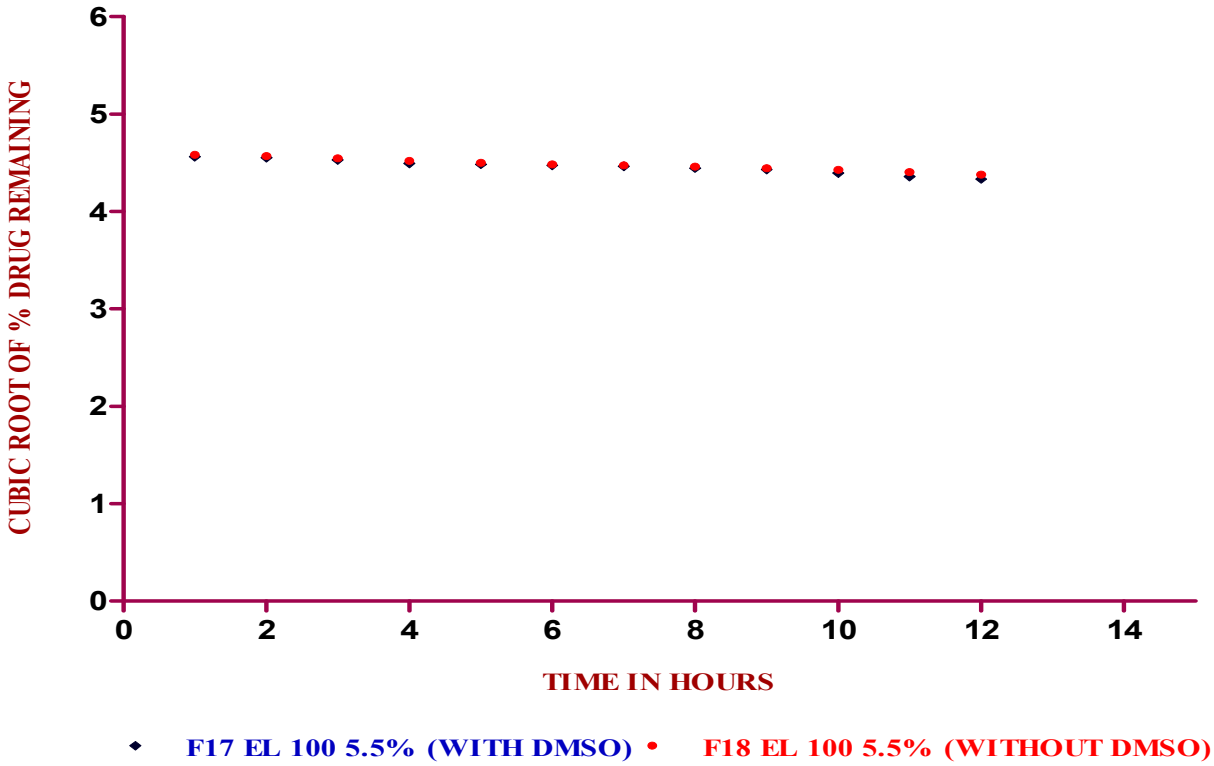
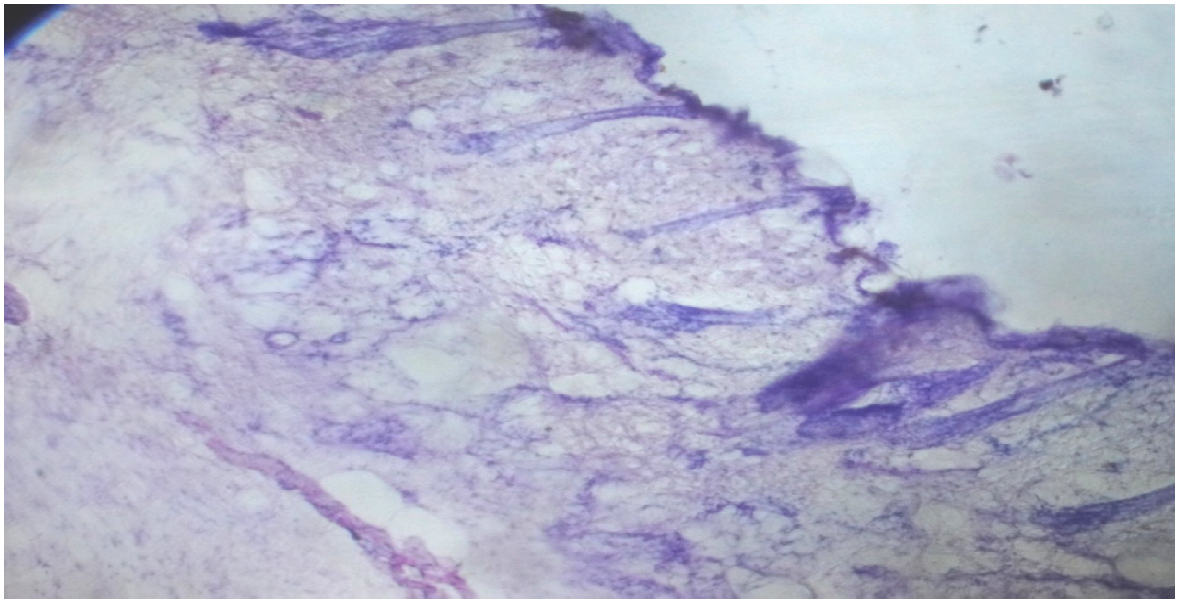


FIGURE 11E COMPARISON OF HIXSON-CROWELL EXVIVO PERMEABILITY RELEASE KINETICS OF FORMULATIONS CONTAINING EUDRAGIT L 100 5.5% (WITH AND WITHOUT DMSO)

A) UNTREATED SKIN



B) SKIN TREATED WITH PHOSPHATE BUFFERED SALINE OF P^{H}7.4

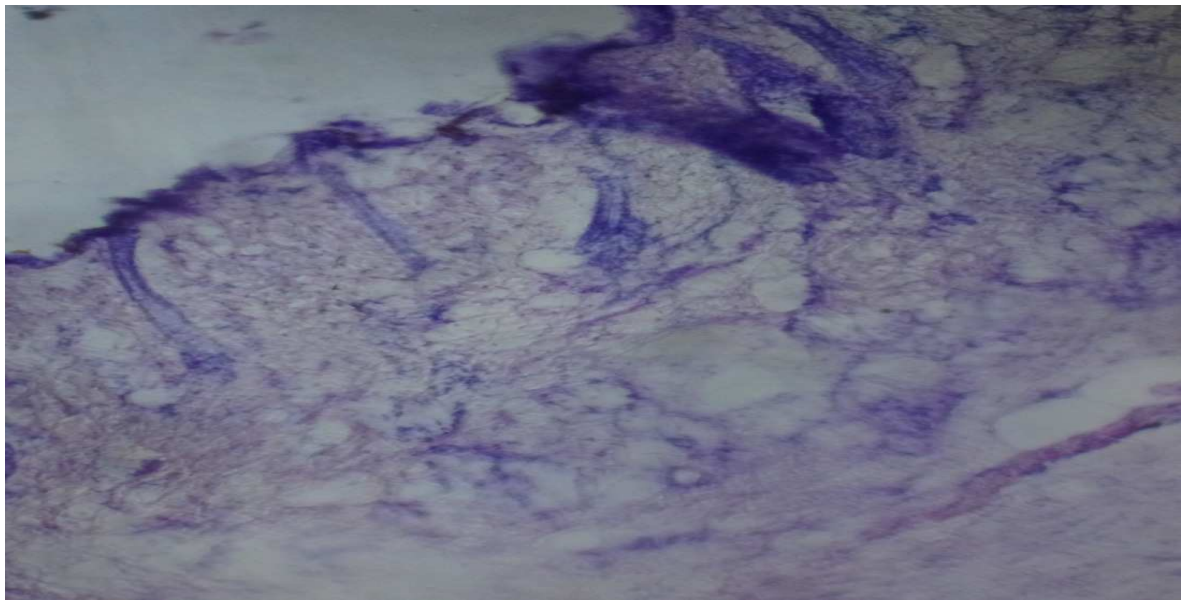
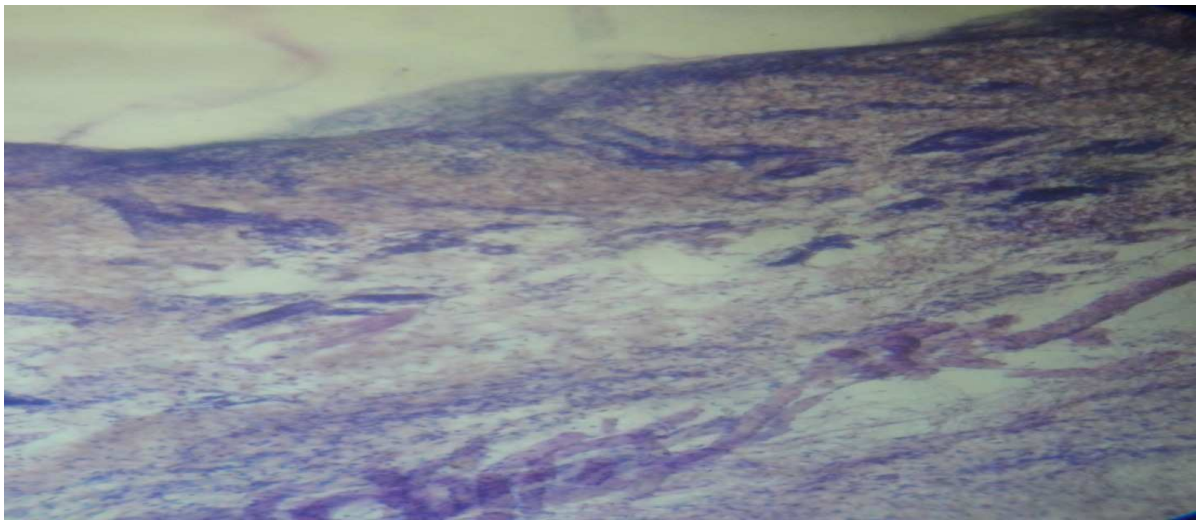


FIGURE 12 HISTOPATHOLOGY OF SKIN SAMPLES ARE AS FOLLOWS

A) UNTREATED SKIN B) SKIN TREATED WITH PHOSPHATE BUFFERED SALINE OF pH 7.4

C) SKIN TREATED WITH BENAZEPRIL HYDROCHLORIDE

PURE DRUG SOLUTION



D) SKIN TREATED WITH TRANSDERMAL PATCH

CONTAINING EL100 5.5% WITH DMSO

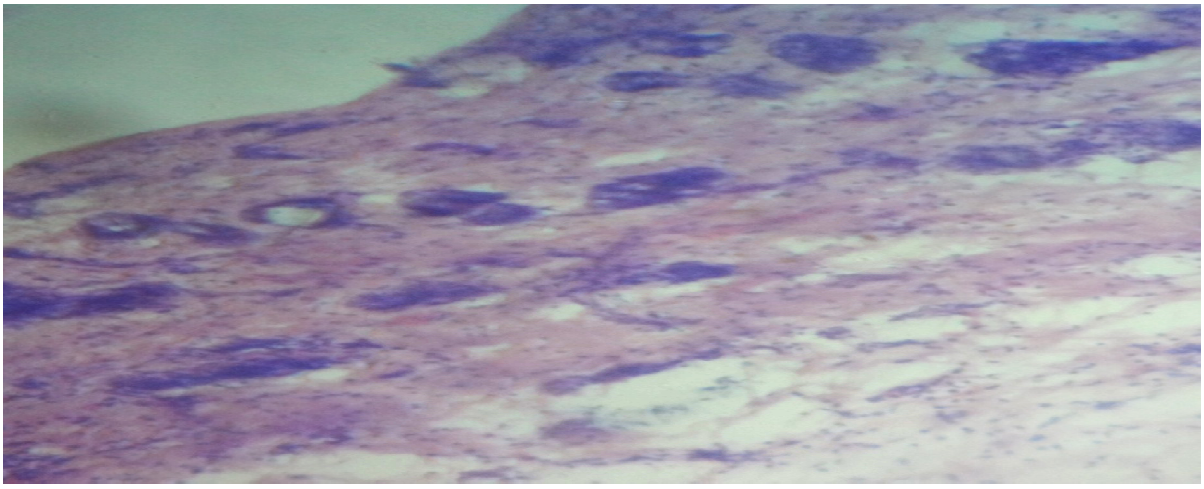


FIGURE 12 HISTOPATHOLOGY OF SKIN SAMPLES ARE AS FOLLOWS

C) SKIN TREATED WITH BENAZEPRIL HYDROCHLORIDE

PURE DRUG SOLUTION D) SKIN TREATED WITH

TRANSDERMAL PATCH CONTAINING EL100 5.5% WITH

DMSO

A) EL100 5.5% WITH DMSO BEFORE *EX VIVO* PERMEATION STUDY

B) EL100 5.5% WITH DMSO AFTER *EX VIVO* PERMEATION STUDY

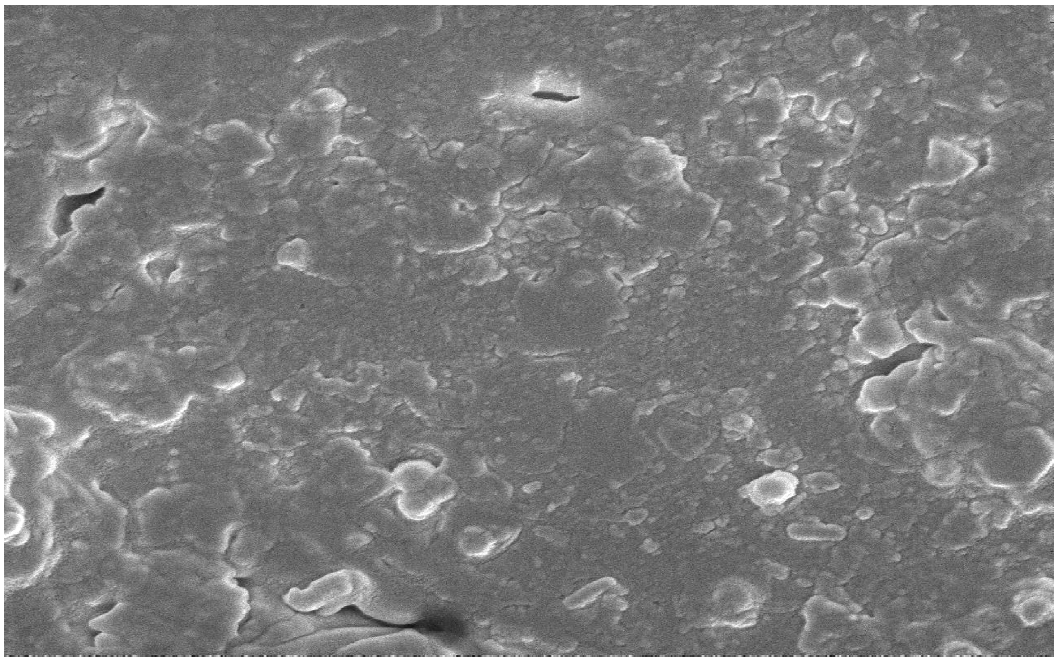


FIGURE 13 SCANNING ELECTRON MICROSCOPY OF THE PATCH CONTAINING EL100 5.5% WITH DMSO ARE AS FOLLOWS A) BEFORE *EX VIVO* PERMEATION STUDY B) AFTER *EX VIVO* PERMEATION STUDY

E) SKIN TREATED WITH TRANSDERMAL PATCH

CONTAINING ES100 5.5% WITHOUT DMSO

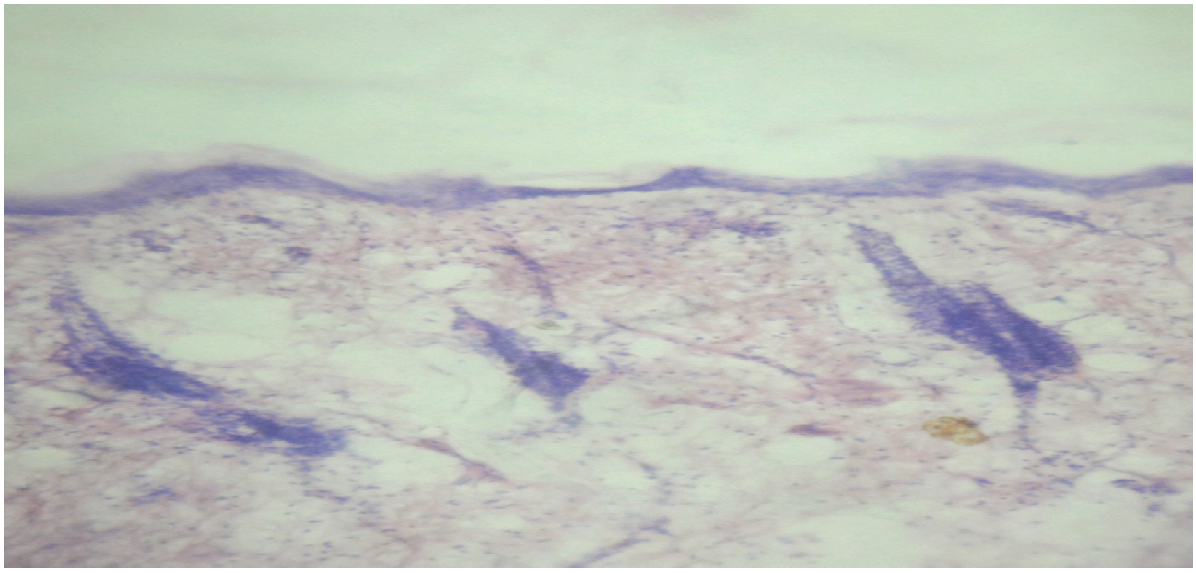


FIGURE 12E HISTOPATHOLOGY OF SKIN SAMPLE

SKIN TREATED WITH TRANSDERMAL PATCH

CONTAINING ES100 5.5% WITHOUT DMSO

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